United States
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Agriculture

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# Fruit Fly Research: 1993 Supplement to the USDA-ARS Action Plan

## First Review
## Beltsville, Maryland
## June 28, 1993

In cooperation with—
USDA/Animal and Plant Health Inspection Service
State Action and Regulatory Agencies
State Agricultural Experiment Stations

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R.M. Faust and J.R. Coppedge, Editors

## CONTENTS

## FOREWORD

The general area of tephritid fruit fly research has become a major focus of many laboratory programs within the Agricultural Research Service (ARS) because of the importance of maintaining the continental United States free of damaging populations of several important pest fruit fly species. In order to develop a comprehensive USDA-ARS Action Plan for Fruit Flies Research, in cooperation with the Animal and Plant Health Inspection Service (APHIS) and the ARS State Agricultural Experiment Station (SAES) cooperators, an organizing committee planned and carried out a major review (San Francisco, July 19 - August 2, 1991) of all ARS research on fruit flies. At this workshop, ARS scientists were requested to present the current status of their work as well as future plans and to provide a written summary of their reports for use by the organizing committee in developing the research action plan. The research action plan was categorized into four major high priority areas for research focus as identified by the action agencies: I. Detection and Delimitation, II. Exclusion, III. Control and Eradication, IV. Fundamental Biology.

Subheadings under each of these major areas were devised, and a team of USDA and APHIS representatives developed the 1991 USDA-ARS Action Plan for Fruit Flies Research based on the input from the ARS scientists as well as the action agency needs that were identified at the workshop.

Since its inception, the USDA-ARS Action Plan for Fruit Flies has provided a basis for monitoring and evaluating program progress in terms of meeting the needs and priorities of the Federal and State action agencies through a periodic review process. A fruit fly action plan progress review was held on June 28, 1993, in Beltsville, MD with a select group of participants representing ARS, APHIS, and seven State agencies (see participant list in Appendix E). A revised list of high priority research areas was defined (see Section VIII). ARS scientists are encouraged to continue developing research plans in concert with Action Agency needs and to continue reporting the results of their studies in a timely manner.

Robert M. Faust
National Program Leader
Fundamental and Molecular Entomology

James R. Coppedge
National Program Leader
Applied Entomology

## ARS/APHIS FRUIT FLY ACTION PLAN ASSESSMENT TEAM

### USDA, ARS, National Program Staff

R. M. Faust, National Program Leader for Fundamental and Molecular Entomology
J. R. Coppedge, National Program Leader for Applied Entomology

| Action Plan Section | ARS Member | APHIS Member |
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| I. Detection and Delimitation | B. A. Leonhardt | M. C. Holmes |
| II. Exclusion | R. L. Mangan | P. J. Gomes |
| III. Control and Eradication | J. L. Sharp | A. S. Green |
| IV. Fundamental Biology | E. B. Jang | D. R. Lance |

### Acknowledgments

The joint ARS-APHIS Fruit Fly Working Group sincerely express appreciation to the members of the Fruit Fly Action Plan Assessment Team and to all the participants for their contributions to the review process and to this document. We especially appreciate the efforts of B. A. Leonhardt and S. L. Dusch for their help in finalization of this report.

## EXECUTIVE SUMMARY

Over the years the USDA has strived to maintain a balanced program on fruit flies and on the management of fruit fly problems in its research, development, education, regulatory and action programs. As the "in-house" research agency for USDA, ARS and its cooperators have accelerated their research efforts on fruit flies in order to better address issues related to (1) detection and delimitation; (2) exclusion; (3) control and eradication; and (4) fundamental biology of the key fruit fly pests. A National Program Plan was developed in the summer of 1991 as a comprehensive, unified effort to develop and implement economically, environmentally sound and publicly acceptable systems for management or eradication of these agricultural pests.

A fruit fly action plan progress review was held on June 28, 1993, in Beltsville, MD with a select group of participants representing ARS, APHIS, and seven State agencies. Individual progress reports as well as the action ares summaries are included in this supplement to the Action Plan. In addition, APHIS progress reports as well as a revised list of program needs are also included in this supplement to the Action Plan.

The objectives of the workshop, in addition to reviewing the progress and the current and proposed research activities in relation to goals, objectives, and priorities, were to (1) make an assessment of how ARS research activities are meeting objectives and action agency needs; (2) identify areas where research is still lacking; (3) reevaluate the relevancy of the needs and priorities as outlined in the 1991 Action Plan, as well as determine what new priorities and needs have emerged, and how best to address them; and (4) provide additional recommendations on specific short- and long-term goals.

Significant accomplishments highlighted during the progress review included: (1) the identification of male-produced pheromonal components; (2) development of a new commercial polymer dispenser for trimedlure; (3) development of successful temperature treatments for a variety of tropical and subtropical fruit for the major fruit fly pests of Hawaii, Florida, and Texas, and for imports from Latin America and the Caribbean basin; (4) the use of gibberellic acid treatments for citrus against Caribfly and Mexican fruit fly; (5) development of an artificial diet for *Anastrepha obliqua*; (6) testing of a pupal color sexing strain of Medfly (males only release) which showed promise in reducing wild fly populations; (7) mass releases of a parasite that resulted in substantial reduction of Caribfly populations; (8) demonstration that pheromone and host fruit odors influence female behavior of Medflies; and (9) development of a food-based dry trap for Medflies.

A list of high priority research needs was generated during the workshop and included: (1) optimization of sterile insect mass rearing; (2) development of methodology to control fruit flies entirely with biologically-based technologies; (3) elimination of dependence on malathion in bait sprays; (4) development and evaluation of a temperature sensitive lethal strain of fruit fly; (5) development of commodity treatments to replace methyl bromide; (6) determination of the precise origin and pathway entry of exotic fruit fly species into the U.S.; (7) development and pilot testing of technologies for the eradication of the four major fruit fly species in Hawaii; (8) development of highly effective attractants and trapping systems for all economically significant fruit fly species; (9) development of fly-free zone technology; and (10) generation of increased knowledge on fruit fly behavior and field biology.

## REVIEW OBJECTIVES

1. Examine the progress as well as the current and proposed research activities in relation to goals, objectives, and priorities as delineated by Federal and State action agencies.

2. Make an assessment of how ARS research activities are meeting objectives and action agency needs.

3. Identify areas where research is still lacking.

4. Provide answers to the questions:

   - Are all the needs and priorities of action agencies as outlined in the Action Plan of 1991 still relevant?

   - Are there new priorities and needs that have emerged and, if so, what are they?

   - If there are new priorities, how can they best be addressed, i.e., what shifts and/or adjustments within the ARS program could or should be considered?

5. Provide recommendations, where appropriate, on specific short- and long-term goals.

# ARS REPORTS OF RESEARCH PROGRESS AND PLANS

## SECTION I. DETECTION AND DELIMITATION

### Research Summary
### Compiled by B. A. Leonhardt & M. C. Holmes

### Pheromones

Several laboratories contributed to pheromone studies of male Mediterranean fruit flies. Pheromonal emissions were trapped, identified (32 components), quantified, and bioassayed. Pheromone production by 5-6 and 20-21 day-old flies peaked in early and late morning, respectively. The most abundant components in 5-12 day old flies were ethyl acetate, 1-pyrroline, ethyl (E)-3-octenoate, geranyl acetate, and (E,E)-α-farnesene. Flight tunnel bioassays were used to delineate the attractiveness of synthetic components. Significantly more factory reared and feral female medflies were attracted to a new multi-component synthetic blend than to the 3-component blend. In field tests, captures of lab-reared virgin female flies with male emission were synergized by addition of green leaf volatiles or some pheromone components. Blends of pheromone compounds failed to attract female flies in coffee farms. Although attractiveness of some pheromone blends has been demonstrated, an effective and reliable mixture does not appear to be ready for use in operational programs.

Male-produced pheromonal components of both *Anastrepha ludens* and *A. suspensa* were identified as: (Z)-3-nonenol, (Z,Z)-3,6-nonadienol, suspensolide, (E,E)-α-farnesene, anastrephin and eplanastrephin; ocimene and bisabolene were also found in *A. suspensa* . The periodicity of pheromone release for both species has been determined. Pheromone compounds can now be formulated to provide a release rate equivalent to that released by males during peak pheromone production. The responses of females to the synthetic pheromone blend and to live males were equal. The identification of the male-produced pheromonal components and periodicity of release were completed for *A. striata, A. obliqua, and A. fraterculus.*

### Parapheromones

Computer modeling showed a correlation of Medfly capture with molecular surface area, molecular volume, torsion angle and interatomic distance for the 8 racemic isomers of trimedlure (TML). Similar correlations are not yet apparent for α-copaene or the highly attractive C isomer of TML; however, structural similarities were found between segments of the MM2-minimized structures. A new commercial polymer dispenser for TML is proving to be an effective alternative to the polymeric plug used in Medfly detection traps. High purity ceralure can now be reclaimed from decomposed samples and protected from changes in isomer content by storage over copper. NMR studies confirmed the stereochemistry of the *trans* isomers of ceralure. Dioxa-spiro compounds were synthesized for Medfly tests. ß-Copaene and ß-yglangene in Angelica seed oil showed attraction to male Medflies but neither of these compounds, or analogs of α-copaene, were as attractive as α-copaene.

Fluorine analogs of methyl eugenol (ME) and several catechols were synthesized for evaluation of oriental fruit fly attraction. Tests were conducted on a promising ethoxy-substituted stereo-isomer of ME. New synthetic methods were developed for α, ß and γ methyl-substituted analogs of ME. A chemical class of compounds was found to be as attractive to *A. ludens* as NuLure.

## Food and Other Attractants

The most important bacteria-produced fruit fly attractants contain nitrogen. Ammonia and 2,5-dimethylpyrazine (DMP) were identified. Ammonia was attractive in lab tests and testing of 2,5-DMP is underway. A mixture of ammonia, methylamine and putrescine was more attractive than Torula yeast in competing traps in a flight chamber. Host lures for *Anastrepha* were tested in Mexico and Guatemala but found to be not competitive with protein baits in orchards with fruit. However, lures on yellow spheres were competitive with Torula yeast in McPhail traps in fruitless orchards. Field trials were conducted to determine the preference of Medflies, Mexflies, and other *Anastrepha* species to aqueous formulations of NuLure at different pH and standard hydrolyzed Torula yeast. Addition of 1-10% borax to 10% NuLure solution increased bait pH which directly correlated with increases in number of female medflies trapped. Preliminary field tests showed a 4-component synthetic mixture was more attractive than NuLure for Mediterranean, melon and oriental fruit flies. Volatiles from Hawaiian fruits were trapped and identified; lab bioassays demonstrated that some volatiles elicit female Medfly attraction and/or oviposition. Extracts of butterfly bush were prepared for evaluation with Hawaiian fruit flies. Bioassay of solvent rinses of coffee berries failed to reveal Medfly attraction. A new cage bioassay was developed for evaluating attractants for *A. ludens* and *A. obliqua*; bioassay of about 95 compounds showed that about 10 of these evoked a response but not as strong as that with the NuLure standard.

## Traps, Devices and Formulations

The amount of borax used with NuLure was reduced by 40% without changing the pH or affecting its attractancy. Torula yeast was found to be as attractive as NuLure. Naled and DDVP in plastic strips were shown to be similarly effective as knock-down toxicants in male lure and hydrolyzed protein bait traps. Ammonia vapor from a slow-release dispenser in a Jackson trap with TML increased trap efficiency for Medfly. Field tests indicated that yellow spheres were good traps for *Anastrepha* but Jackson traps were not. Yellow panels gave mixed results.

## Systematics

Differences in the 16S ribosomal RNA genes (rDNA) of bacteria found in medflies were sought for distinction of geographic populations. Specific primers were used to amplify 16S rDNAs using the polymerase chain reaction (PCR). Two of the 14 restriction enzymes examined yielded restriction fragment patterns (RFLPs) which distinguished certain, but not all, Medfly populations by geographic origin. Distinction on the basis of the host-plant was observed.

RFLP's were used to survey 15 established natural populations and 5 laboratory cultures from Hawaii, Central and South America, West Africa, and recent California infestations. Restriction enzyme cleavage sites varied among populations; Hawaii was found to be an unlikely source for the 1989 and 1991 California infestations. Samples from over 50 populations were analyzed to create a restriction map for the Medfly mtDNA. A PCR procedure is being developed to identify mtDNA haplotypes from archived specimens. Variation in genomic DNA was found using PCR and randomly amplified polymorphic DNA (RAPD) primers. All California flies were found to possess a 625 base pair fragment that also occurred in 19 of 34 populations tested.

Clones of the 2nd internal transcribed spacer (ITS2) of the Medfly RNA transcript were repeated from various populations. The clones will be used to determine the intra-strain homogeneity in the ITS2 nucleotide sequences. Sequences of approximately 200 bases each from the flanking ends of two strains revealed no mismatches of nucleotide. Nucleotide sequencing of the more variable, central portion of the ITS2 will require the synthesis of new primers.

A prototype of the Expert System for identification of economically important fruit flies was completed. The prototype runs on MS-DOS personal computers, covers some 80 species, uses

some 182 characters, and includes illustrations for 43 characters and 56 species, with help screens and help text. The morphology of immature stages of 4 fly species were examined to find taxonomic keys. Descriptions of two new species of *Anastrepha* were published. Initial morphological analysis of the *fraterculus* complex was unsuccessful. Revision of the fruit fly fauna of Mexico continued with study of the species of *Trypeta* and *Eutreta*. The Handbook of the Fruit Flies (Diptera: Tephritidae) of America North of Mexico was completed.

### Semiochemical Structure/Activity Relationships

Single-cell electrophysiological recordings were initiated on Medfly adults but sufficient time for olfactory stimulation cannot as yet be maintained. Analogs of ME showed electroantennogram (EAG) responses with oriental fruit fly that suggested molecular affinity to receptors may be important in structural activity relationships. Isomers of ceralure await EAG studies.

### Behavior

Some low boiling compounds (including ammonia) identified from vegetable protein hydrolysate were attractive to Medflies. Addition of ammonia to TML increased Medfly attraction. Ammonia was attractive to female *Rhagoletis completa*. Nine species of bacteria identified from wild *A. ludens* were attractive to flies with one as, or more, attractive in field tests than protein bait. In lab tests, fruit and plant odors showed behavioral responses of fruit flies. Papaya fruit flies were shown to multiple mate in the presence of papaya fruit but singly mate in the absence of fruit. Mediterranean and Caribbean fruit flies were shown to depend on sugar feeding to maintain pheromonal calling.

Investigators Name: **James W. Avery**
Affiliation and Location: **ICEL, PSI, Beltsville, MD**
Research and Implementation Area: **I.B.1.d, I.B.7**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **1,2,70**

**SUMMARY:**

**I.B.7** A method was developed to determine the residual concentration of Mediterranean fruit fly attractants, trimedlure and ceralure, in stickem and in environmental samples. Extracts of coffee leaves and berries as well as of surrounding soil were analyzed for trace levels of the attractants but no residual attractants were detected in these extracts. The results of the report submitted to EPA resulted in an EUP for further field tests.

**FY 93 WORK PLANS:**

**I.B.1.d** New methods for the stereospecific synthesis of insect attractants for Mediterranean fruit fly, trimedlure and ceralure, are in progress. If successful, this method will allow the more effective, stereospecific isomers to be used in detection traps.

Investigator's Name: **Carroll O. Calkins**
Affiliation and Location: **IABBBRL, Gainesville, FL**
Research and Implementation Areas: **I.C.2.a**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **7,8,9,51,54,69,118,126,142**

**SUMMARY:**

**I.C.2.a** Experiments were developed, in cooperation with Robert Heath, to elucidate the attractive components of torula and hydrolyzed yeasts used as attractants for the Caribbean fruit fly. It was determined that female Caribbean fruit flies that are 5 days of age are most responsive to torula yeast in McPhail traps. Flies are most responsive to food baits during the middle of the day when light intensity is the greatest.

**FY 93 WORK PLANS:**

**I.C.2.a** Field tests to confirm the attractiveness of components of food baits are presently underway.

Investigator's Name: **Bruce C. Campbell, Gloria Merrill**
Affiliation and Location: **WRRC, Albany, CA**
Research and Implementation Areas: **I.F.1, I.F.4**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A):

**SUMMARY:**

**I.F.1** A study was conducted to determine if differences in the 16S ribosomal RNA genes (rDNA) of bacteria found in Mediterranean fruit flies (Medflies) could be used to distinguish geographic populations. Specific primers were used to amplify 16S rDNAs

of the Medfly bacterial flora using the polymerase chain reaction (PCR). After amplification, subsamples of the PCRed 16S rDNAs were individually digested with the following restriction enzymes: *Hph* I, *Alu* I, *Bam* HI, *Bgl* II, *Cla* I, *Eco* RI, *Eco* RV, *Hind* III, *Pst* I, *Sac* I, *Taq* I, *Dra* I, or *Pvu* I. Restriction fragment patterns RFLPs) of these digests were examined on 1.5% agarose and 8% polyacrylamide gels and compared between various Guatemalan and Hawaiian Medfly populations. Of the 14 restriction enzymes examined, *Hph* I and *Alu* I yielded RFLPs which distinguished certain Medfly populations. There were numerous homologous bands in all populations of Medflies indicating the presence of a common bacterium or bacteria. Bands were distinctive on the basis of the host-plant from which the fly was collected.

**I.F.4** Clones of the second internal transcribed spacer (ITS2) of the Medfly RNA transcript (approx. 700 bp in size) were prepared from the following Medfly populations: 1. Hawaiian lab strain used in mass-rearing of sterile males, provided by Eric Jang, 2. Kauai strain VI, P-gen, provided by Don McInnis, 3. Mauna Loa strain VII, P-gen, provided by Don McInnis, 4. Hawaiian sexing strain $R^2Pa$, provided by Don McInnis, and 5. Sacatepequez Guatemala Medflies from coffee, provided by Ken Bloem and Derrell Chambers. Numerous clones (>10) from each strain will be used to determine the amount, if any, of intra-strain heterogeneity. Sequences of approximately 200 bases, each, from the flanking ends of the ITS2 of singular clones on an Hawaiian lab strain and a Sacatepequez strain revealed no mismatches of nucleotides. We are now sequencing the more variable, central portion of the ITS2s. In a recent study (Campbell *et al* ., unpublished material) it was found that the sibling species complex of the pteromalid wasp genus *Nasonia* possessed only one to two mismatches in the flanking region with a dozen to two dozen mismatches in the central portion of the ITS2s. No work was initiated to distinguish host-plant races of *Urophora sirunaseva* or *Chaetorellia australis.*

## FY 93 WORK PLANS:

**I.F.1,4** Research will continue on sequencing the ITSs of Medflies in order to determine if this region of the RNA transcript can be used for identification or elucidation of phylogenetic affiliations of Medfly geographic races. Research will be initiated on cloning and sequencing the genes of mitochondrial DNAs of Medflies to determine if this region can identify geographic races of Medflies. A similar effort using the ITSs and mtDNAs of *Urophora* or *Chaetorellia* is also anticipated.

Investigator's Name: **Roy T. Cunningham**
Affiliation and Location: **TFVRL, Hilo, HI**
Research and Implementation Areas: **I.B.2.b, I.C.1.a**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **1,2,13,63,64,70,76,143,144**

**SUMMARY:**

**I.B.2.b** Purer preparation of a promising ethoxy-substituted stereoisomer of methyleugenol was tested. Also, a new series of fluorine substituted analogs showed some promise in preliminary field testing.

**I.C.1.a** Further testing of oligocomponent mixtures at higher concentrations showed greatly increased attraction for all species. Testing of certain new components in mixtures also showed promise as substitutes for protein hydrolysates.

**FY 93 WORK PLANS:**

**I.B.2.b** Larger quantities of the 5 or so most promising analogs should be tested in standard survey traps with the standard doses in order to evaluate their performance as substitutes for methyleugenol in trap detection arrays. Preliminary work with slow-release dispensers for the more volatile analogs will be started.

**I.C.1.a** Cooperative testing at higher concentrations and at different ratios of components will be evaluated. Use of these components to enhance the protein hydrolysates will be tested.

Investigator's Name: **Albert B. DeMilo**
Affiliation and Location: **ICEL, Beltsville, MD**
Research and Implementation Areas: **I.B.1.b, I.B.1.c, I.B.1.d, I.B.2.b, I.B.5.a**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **2,12,13,14,63,64,143,144**

**SUMMARY:**

**I.B.1.b** Determined that high purity ceralure could be reclaimed from decomposed samples by adding hydroquinone and Irganox during distillation and that ceralure distillates stored 3-4 months over copper were stable with only minor changes in isomer content and ratio.

**I.B.1.c** Completed modeling of α-copaene with CHEM-3D PLUS. Structural similarities were found between segments of the MM2 minimized structure of α-copaene and minimized TML-C and ceralure B1 isomers.

**I.B.1.d** Completed 2-D NMR studies to confirm stereochemistry of the four trans isomers of ceralure. Synthesized six dioxa-spiro compounds for Medfly tests. Synthesized a variety of catechols as potential alternatives to methyl eugenol.

**I.B.2.b** Developed methods to synthesize α, ß and γ methyl-substituted analogs of methyl eugenol (ME). Structure-activity correlations were identified from 19 synthesized

analogs; several were highly attractive and more persistent than ME. Synthesized 6 fluorine analogs of ME, several of which are appreciably attractive in field tests.

**I.B.5.a** Sixty-six compounds were evaluated as candidate Mexican fruit fly attractants. Several compounds from a single chemical class were found more attractive than NuLure. Twelve extracts of grapefruit were prepared, tested, but found unattractive.

## FY 93 WORK PLANS:

**I.B.1.b** Evaluate other free radical inhibitors. Determine effect of inhibitors on attractancy. Monitor ceralure samples preserved with various inhibitors over a 6-12 month period.

**I.B.1.c** Select, synthesize and evaluate cyclic compounds for attractancy, structures of which are similar to α-copaene models.

**I.B.1.d** Continue synthesis of TML analogs with emphasis on modification (i.e., bulky non-halogenated groups at the 1, 4 and 5 positions of the ring). Synthesize additional oxa- and dioxaspiro compounds and continue structure-activity studies.

**I.B.2.b** Continue synthesis of ME analogs with emphasis on fluorine substitution on the allyl moiety; determine toxicology of promising analogs.

**I.B.5.a** Continue synthesis and field evaluation of analogs of promising attractants identified in FY 92.


| | |
|---|---|
| Investigator's Name: | **Robert A. Flath** |
| Affiliation and Location: | **WRRC, PWA, Albany, CA** |
| Research and Implementation Area: | **I.A.1, I.B.1.c, I.B.4.a, I.C.2.b, I.C.2.c** |
| Dates Covered by Report: | **10/1/91 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **17,71,112,130** |


## SUMMARY:


**I.A.1** Male Medfly emissions were trapped and the components identified (thirty-two) and semiquantified. Three fly ages were used (5-6 day, 11-12 day, and 20-21 day). Early, mid- and late morning samples were collected from the first two age groups. Ethyl acetate, 1-pyrroline, ethyl (E)-3-octenoate, geranyl acetate and (E,E)-alpha-farnesene were the most abundant components from the first two age groups. Releases peaked in: early morning (5-6 day), mid- to late morning (11-12 day), and late morning (20-21 day). Such quantitative and qualitative information is necessary in efforts to formulate an effective and practical pheromonal blend for virgin female fly attraction.

**I.B.1.c** Two additional sesquiterpenes attractive to the male Medfly were identified in Angelica seed oil. These were beta-copaene [61.4%(+), 38.6%(-)] and beta-ylangene [91.9%(+), 8.1%(-)]. Neither is as attractive as alpha-copaene [98.6%(+), 1.4%(-)] from the same oil. A number of sesquiterpenes related to alpha-copaene were also obtained or isolated from other oils and bioassayed, to investigate the relationship between (+)-alpha-copaene's structure and attractiveness. None of the analogs are as attractive to the male Medfly as (+)-alpha-copaene. This work is part of an ongoing

effort to find effective male lures, and to better understand the structure-activity relationships of alpha-copaene, a potent attractant for the male Medfly.

**I.B.4.a** No progress on latilure analogs.

**I.C.2.b and I.C.2.c.** Volatile components from several Hawaiian fruit fly host plants were concentrated, fractionated, and examined by GC/MS. Bioassays indicate some female fly attraction. Additional work is needed to determine whether any useful female attractants can be identified in these plant sources.

## FY 93 WORK PLANS:

**I.A.1** No further compositional studies for the present.

**I.B.1.c** Some effort to locate economic sources for (+)-alpha-copaene and/or the more attractive analogs.

**I.B.4.a** Contingent on scheduling efforts with cooperators.

**I.C.2.b and I.C.2.c.** Several host plants will be examined in more detail.


| | |
|---|---|
| Investigator's Name: | **Robert R. Heath** |
| Affiliation and Location: | **IABBBRL, Gainesville, FL** |
| Research and Implementation Areas: | **I.A.1, I.A.2.a, I.A.2.b, I.C.2.a** |
| Dates Covered by Report: | **10/1/91 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **3,4,15,16,51,52,53,54,55,67,68, 122,123** |


## SUMMARY:


**I.A.1** A flight tunnel bioassay system was used to delineate the attractiveness of synthetic pheromonal components to female Medflies. Significantly more factory reared and feral female Medflies were attracted to the new multi-component synthetic blend in comparison with the attraction to the previously published three component blend.

**I.A.2.a** The identification of the pheromonal components and periodicity of release has been completed. The compounds that have been identified are: (Z)-3-nonenol, (Z,Z)-3,6-nonadienol, suspensolide, E,E-α-farnesene, anastrephin and epi-anastrephin.

**I.A.2.b** The identification of the pheromonal components and periodicity of release has been completed. The compounds that have been identified are: (Z)-3-nonenol, (Z,Z)-3,6-nonadienol, ocimene, bisabolene, suspensolide, E,E-α-farnesene, anastrephin and epi-anastrephin. We have developed a method to formulate these compounds to provide a release rate equivalent to that released by males during peak pheromone production. The response of females to the synthetic pheromone blend in flight tunnel bioassays was equal to the response to live males in side-by-side comparisons.

**I.A.2.b** The identification of the male produced pheromonal components and periodicity of release has been completed for *A. striata, A. obliqua,* and *A. fraterculus.*

**I.C.2.a** Field trials were conducted to determine the preference of Medflies, Mexflies, and other *Anastrepha* species to aqueous formulations of the protein bait lure NuLure at different pH and standard hydrolyzed torula yeast pellets. Addition of 1-10% borax to 10% Nu-Lure solution increased bait pH, and this increase was directly correlated with increase in number of female Medflies trapped in field trials conducted in Guatemala. The percent of female Medflies trapped with NuLure at a pH of 8.6 was significantly greater than traps baited with five hydrolyzed torula yeast pellets. NuLure plus 1% borax caught significantly less female and male Medflies and Mexflies than either torula yeast pellets or NuLure containing 3, 5, or 10% borax.

## FY 93 WORK PLANS:

**I.A.1, I.A.2.a, I.A.2.b**
Develop pheromone-based system to monitor and suppress populations of Medflies and *Anastrepha* species.

**I.C.2.a** Identify attractive chemicals, formulate chemicals and develop a food-based dry trap.


| | |
|---|---|
| Investigator's Name: | **Eric B. Jang** |
| Affiliation and Location: | **TFVRL, Hilo, HI** |
| Research and Implementation Areas: | **I.A.1, I.G, I.H** |
| Dates Covered by Report: | **10/1/91 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **10,17,60,61,62,71,88** |


## SUMMARY:


**I.A.1** Attractancy of volatile pheromonal male odors to females of Mediterranean fruit fly, melon fly and oriental fruit fly has been established in the lab. Major, intermediate and minor components of Medfly pheromone have been identified and bioassayed to show attractiveness of certain compounds. Effects of time of day on emissions has been studied.

**I.G** EAG responses of oriental fruit fly to analogs of methyl eugenol suggest that molecular "affinity" to receptors may be important in structure-activity relationships. No apparent difference between males and females were found. Differential adaptation studies were initiated. Single cell recordings of Medfly antennal receptors were also looked at.

**I.H** The behavioral responses of fruit flies to host fruit/plant odor has been studied in the lab using plant parts and extracts. Several bioassays suggest that host plants may contain compounds attractive to flies depending on their physiological state. Further studies are, however, needed to identify specific compounds.


## FY 93 WORK PLANS:

**I.A.1** Field tests of major, intermediate and minor components will be completed. Further laboratory studies will be initiated to assess the effects of physiology on pheromone responses of flies.

**I.G** EAG and differential adaptation studies will be completed. Single cell recordings of Medfly receptors will continue. EAG's of Malaysian fruit flies will be initiated.

**I.H** Behavioral responses of fruit flies will continue, primarily in the laboratory. At least one extract will be tested in outdoor field cages. Basic observations on fruit fly behavior will continue as information in this area is scarce.


Investigator's Name: **Peter J. Landolt**
Affiliation and Location: **IABBBRL, Gainesville, FL**

Research and Implementation Areas: **I.A.1, I.A.2.a, I.H, III.B.2.b**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **51,54,55,65,66,67,68**

## SUMMARY:


**I.A.1** Multiple component Mediterranean fruit fly pheromone blends were field tested in coffee farms as trap baits with no evidence of attraction.

**I.A.2.a** Bioassay conditions were determined for good female Caribbean fruit fly attractive responses to males and to odors of males.

**I.H** It was found that papaya fruit flies mate multiply in the presence of papaya fruit, and singly in the absence of papaya fruit. Mediterranean and Caribbean fruit flies are dependent on sugar feeding to maintain pheromonal calling.

**IV.A.4** Patterns of feeding by *Anastrepha suspensa* on sucrose and yeast were documented as a function of time of day and fly age, and correlated to fly reproductive development and sexual activity.


## FY 93 WORK PLANS:

**I.A.1** Additional pheromone components will be considered for testing as part of multicomponent blends.

**I.A.2.a** None planned.

**I.H** Effects of host odor and oviposition in host fruit on papaya fruit fly mating frequency will be determined.


Investigator's Name: **Barbara A. Leonhardt**
Affiliation and Location: **ICEL, PSI, Beltsville, MD**

Research and Implementation Area: **I.A.3, I.B.7, I.F.4**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **1,2,14,64,70**

## SUMMARY:


**I.A.3** No progress was made on the isolation and identification of the pheromone of *Dacus latifrons*. A bioassay needs to be developed and fresh insect extract needs to be

compared with previously analyzed material. This project will be renewed by James Avery in August 1993.

**I.B.7** A new commercial polymeric dispenser for trimedlure has been field tested and analyzed chemically. Thus far, it is as, or more, effective than the standard plug dispenser now used in detection traps for Medfly. A grant proposal was funded under the IPM System Evaluation Program for the development of polymer dispensers for attractants of fruit flies other than Medfly.

**I.F.4** No research was conducted on the use of cuticular wax profiles of Medflies to try to identify their origin.

**FY 93 WORK PLANS:**

**I.A.3** An effective bioassay for determining response of *Dacus latifrons* will be sought and fresh extracts and collected volatiles from male flies will be analyzed.

**I.B.7** Polymeric dispensers for methyl eugenol, latilure and ceralure will be developed.

**I.F.4** No research planned.


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| Investigator's Name: | **Douglas M. Light** |
| Affiliation and Location: | **WRRC, PWA, Albany, CA** |
| Research and Implementation Areas: | **I.A.1, I.C.2.b, I.C.2.c, I.G, I.H** |
| Dates Covered by Report: | **10/1/91 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **17,62,71,134** |


**SUMMARY:**


**1.A.1** Pheromonal emissions of male Medflies were trapped and the effects of time of calling and age were analyzed. Identified intermediate and minor components synergized the attractancy of the natural male odor. Similarly, green leaf volatiles had a significant synergistic effect on capture of lab-reared virgin female Medflies.

**I.C.2.b,c** Volatile components from several Hawaiian host fruits were trapped and chemically analyzed. Preliminary, multiple-choice, lab bioassays have demonstrated that certain volatiles elicit attraction and/or oviposition in Medflies.

**I.G** Single-sensillum ("single-cell") electrophysiological recordings have been initiated and continue to be attempted on Medfly adults. But successfully recording and "holding of a cell" for a time interval long enough to complete olfactory stimulations has eluded our efforts.

**I.H** See Teranishi *et al.*


**FY 93 WORK PLANS:**

**I.A.1** Continuation of behavioral bioassays to resolve the putative pheromonal agents and host plant synergists for Medflies.

**I.C.2.b,c** Continuation of plant volatile bioassays on Medflies, and expansion to *Bactrocera* species, might be undertaken by the project's cooperator, Eric Jang.

permit.

**I.H** Behavioral and electrophysiological bioassays will be conducted on both Medflies and the walnut husk fly to investigate attractants from protein hydrolysate.


Investigator's Name: **Nicanor J. Liquido**
Affiliation and Location: **TFVRL, Hilo, HI**

Research and Implementation Areas: **I.A.1, I.A.3, I.B.2.b, I.C.1.a, I.D.1, I.F**

Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **63,72,73,74,75,76,77,78,79**

## SUMMARY:


**I.C.1.a** Based on preliminary field experiments, a four-component synthetic mixture showed a higher attractancy to Mediterranean fruit fly, melon fly, and oriental fruit fly than the standard 'NuLure'.

**I.D.1** A 40% reduction in the amount of borax of the standard hydrolyzed protein bait was attained without changing the pH or affecting the attractancy of the mixture. Torula yeast was found to be as attractive as the standard hydrolyzed protein bait. Naled (15%) in plastic strip was found to be as effective as DDVP(7%) (+ 9% O-isoproxyphenyl methylcarbamate) also in plastic strip as a knock-down toxicant in male lure and hydrolyzed protein bait traps. Ammonia vapor from a slow release dispenser placed inside a Jackson trap with trimedlure increased the trap efficiency for Mediterranean fruit fly.

**I.F** The morphology of eggs, larvae, and pupae of Mediterranean fruit fly, melon fly, Malaysian fruit fly, and oriental fruit fly was studied with the objective of finding taxonomic characters that can be used in developing keys for the identification of the immature stages of these species.


## FY 93 WORK PLANS:

**I.A.1,3** Initiate research that will determine the effect of age and mating status on the pheromone production by Malaysian fruit fly.

**I.B.2.b** Using wild populations, validate the attraction of promising analogs of methyl eugenol to oriental fruit fly males. The performance of these analogs as substitutes for methyl eugenol will be tested using the standard survey trap with standard lure dose in various trap detection arrays.

**I.C.1.a** Continue formulating chemically defined hydrolyzed-protein-bait-based lure.

**I.D.1** Continue studies that aim to compare the efficiency of all traps (wet and dry) that are currently being used in fruit fly detection and survey programs.

**I.F** Finish the morphometric and scanning electron micrograph analysis. Develop keys that are useful in identifying immature stages of these flies.

Investigator's Name: **M. S. Mayer**
Affiliation and Location: **IABBBRL, Gainesville, FL**
Research and Implementation Areas: **I.G**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A):

**SUMMARY:**

**I.G** No EAG correlations with behavioral responses have been obtained.

**FY 93 WORK PLANS:**

**I.G** No work is planned for FY 93 pending development of chemicals and behavioral assays.

Investigator's Name: **Donald O. McInnis**
Affiliation and Location: **USDA,ARS, Honolulu, HI**
Research and Implementation Areas: **I.F.1**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **50,86,87**

**SUMMARY:**

**I.F.1** Genetic variation in tephritid populations, especially of the Medfly, *Ceratitis capitata*, was monitored using DNA fingerprinting techniques in collaboration with Dr. David Haymer of the Univ. of Hawaii (Manoa). The incumbent also received training on various molecular techniques involving DNA fingerprinting. Fly samples from Hawaii, both wild and mass-reared, were analyzed along with captured flies from California.

**FY 93 WORK PLANS:**

**I.F.1** Further analysis is needed from fly samples around the world in order to try to determine likely sources of infestation for outbreaks of Medflies on the U.S. mainland.

Investigator's Name: **Daniel S. Moreno**
Affiliation and Location: **SARL, Weslaco, TX**
Research and Implementation Areas: **I.B.5.a,b**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **81,109**

**SUMMARY:**

**I.5.B.a,b** We modified a room to control temperature, humidity and lighting. We constructed a two-cubic-meter aluminum cage to contain flies for bioassays. We worked out the methodologies of handling flies for testing, feeding, presentation of attractants and determined age structure of flies for testing. We standardized a bioassay procedure using 30% Nu-Lure as the attracting standard. We found that *A. obliqua* requires higher illumination during testing than *A. ludens*. We determined that we can test

most any time during the day but to minimize problems with fly response, we exhaust air continually during testing and at least one hour between tests. Our standard testing methods have proven reliable several times. We have tested ≈ 95 compounds; of these, 10 evoked a response similar to our standard. However, none have been better than the Nu-Lure standard.

## FY 93 WORK PLANS:

**I.B.5.a,b** We plan to continue testing potential Mexican and West Indian fruit fly attractants as they become available during the year.

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| Investigator's Name: | **Allen L. Norrbom** |
| Affiliation and Location: | **SEL, Washington, D.C.** |
| Research and Implementation Areas: | **I.F.4** |
| Dates Covered by Report: | **10/1/91 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **18,104,135** |

## SUMMARY:

**I.F.4** Descriptions of two new species of *Anastrepha* were published. Initial morphological analysis of the *fracterculus* complex was unsuccessful. No characters could be found to diagnose populations that appear to be cryptic Species based on isozyme analysis. Further analysis will be conducted. Revision of the fruit fly fauna of Mexico continued, with study of the species of *Trypeta* and *Eutreta* (collaboration with H.-Y. Han and V. Hernandez). The genus *Epochra*, which includes a pest of currants, was discovered to be the same as (a synonym of) the Old World genus *Euphranta* subgenus *Rhacochlaena*. A new species from Mexico was described. The Handbook of the Fruit Flies (Diptera: Tephritidae) of America North of Mexico (coauthored with R.H. Foote and F.L. Blanc) was completed and published July, 1993. It includes identification keys, illustrations, distribution maps and host plant data for all 300 species known from Canada and the United States.

## FY 93 WORK PLANS:

**I.F.4** The *Anastrepha pallidipennis* complex, which includes pests of *Passiflora*, is being studied. A newly discovered character (thoracic microtrichial patterns) has allowed diagnosis of several species. Revision of the genera *Cryptodacus, Haywardina* and *Rhagoletotrypets* (subtribe Carpomyina) was completed. They are closely related to *Rhagoletis*, which includes numerous pest species, and were studied to improve the classification and identification of the entire subtribes.

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| Investigator's Name: | **David C. Robacker** |
| Affiliation and Location: | **SARL, CQFIR, Weslaco, TX** |
| Research and Implementation Areas: | **I.A.2.a, I.C.1.a, I.C.2.a, I.D.2, I.D.4, I.H** |
| Dates Covered by Report: | **10/1/91 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **108,109,110,111,112,113** |

## SUMMARY:

**I.A.2.a** No work was done because synthetic pheromones were not available.

**I.C.1.a** Evidence indicated that the most important bacteria-produced attractants contain nitrogen. Ammonia and 2,5-dimethylpyrazine were identified (R. Flath, R. Heath). Ammonia was attractive in lab tests. Testing of 2,5-DMP is underway. A mixture of ammonia, methylamine and putrescine was more attractive than Torula yeast in competing traps in a flight chamber. This 3-component mixture has potential for use in a dry trap to replace protein baits in McPhail traps.

**I.C.2.a** Field tests of host fruit derived lures were conducted in Mexico and Guatemala (K. Bloem & S. Bloem). Lures were not competitive with protein baits in orchards with fruit. Lures on yellow spheres were competitive with Torula yeast in McPhail traps in fruitless orchards. These results suggest that effectiveness of baits depends on environmental conditions as well as inherent attractiveness.

**I.D.2** Field tests indicated yellow spheres were good traps. Jackson traps were worthless. Yellow panels gave mixed results.

**I.D.4** No tests were conducted because synthetic pheromones were not available and bacterial attractants have not been completely identified or tested.

**I.H** Nine species of bacteria identified from wild *A. ludens* (A.J. Martinez) were attractive to flies in lab tests. The most attractive bacterium was at least as attractive as protein baits in field tests.

## FY 93 WORK PLANS:

**I.A.2.a** Nothing is planned.

**I.C.1.a** Identification and testing of bacteria-produced chemicals will continue. The ammonia/methylamine/putrescine mixture will be field tested.

**I.C.2.a** More field tests of lures will be conducted.

**I.D.2** More field tests with yellow spheres will be conducted.

**I.D.4** Field tests of host lures combined with nitrogenous attractants are planned.

**I.H** More bacteria species will be field tested (A.J. Martinez).

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| Investigator's Name: | **W.S. Sheppard, G. Gasparich (B.A. McPheron, G.J. Steck)** |
| Affiliation and Location: | **BRL, Beltsville, MD (Penn State Univ, FL Dept of Plant Indus.)** |
| Research and Implementation Areas: | **I** |
| Dates Covered by Report: | **10/1/91 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **120,129** |

## SUMMARY:


I Developed procedures to analyze restriction fragment length polymorphism (RFLP) in the Medfly. A survey of 15 established natural populations and 5 laboratory cultures from Hawaii, Central America, South America, West Africa and samples of recent California infestations was conducted. Restriction enzyme cleavage sites were found to vary among populations and, based on RFLP data from two enzymes, Hawaii was found to be an unlikely source for the 1989 and 1991 California infestations (Sheppard et al., 1992). Subsequently, samples from over 50 populations, representing 12 countries have been collected for analysis.

A restriction map for the Medfly mtDNA has been created and is being used to develop a polymerase chain reaction (PCR)-based procedure to identify mtDNA haplotypes from archived (pinned or alcoholic) specimens.

Variation in genomic DNA was found using PCR and randomly amplified polymorphic DNA (RAPD) primers. All California flies were found to possess a 625 base pair (bp) fragment that also occurred in 19 of 34 populations tested.


## FY 93 WORK PLANS:

I The basic research plan consists of 1) continued collection and screening of potential source populations of known genetic markers and 2) substantial effort to identify new genetic markers to further characterize California infestations and delineate among potential foreign source populations.

1) Collections from additional New and Old World sites are ongoing and planned. A trip to obtain samples from natural populations from Argentina and Brazil is underway (March 1993). Contingent on funding by APHIS, we will conduct a similar trip to the Mediterranean region in the fall of 1993.

2) All incoming samples will be screened for known genetic markers (both mitochondrial and genomic DNA). With the known markers (mtDNA and RAPD), as sample sizes from potential source populations increase, so will our ability to exclude some of them as sources for U.S. infestations. However, to discriminate among countries sharing current genetic markers with California, further markers are required and FY 93 research will include surveying additional restriction enzymes, screening additional RAPD primers, and a search for genetic variation in nuclear molecules, including ribosomal DNA spacers and gene intron and spacer regions.

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| Investigator's Name: | **John M. Sivinski** |
| Affiliation and Location: | **IABBBRL, Gainesville, FL** |
| Research and Implementation Areas: | **I.A.2.b** |
| Dates Covered by Report: | **10/1/91 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **5,51,54,55,66,121,122,123,124,125, 126** |

**SUMMARY:**

**I.A.2.b** Funds were obtained from the Florida Citrus Commission to support the work of Bob Heath on development of new bait and pheromone lures. Further assistance in the form of a supply of wild flies and organization of experiments in the field was provided.

**FY 93 WORK PLANS:**

**I.A.2.b** Attempts will be made to aid in obtaining further funds, and assistance in the field will be provided.

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| Investigator's Name: | **R. S. Teranishi, S. Kint, D.M. Light** |
| Affiliation and Location: | **WRRC, Albany, CA** |
| Research and Implementation Areas: | **I.C.1.a, I.C.1.C, I.H** |
| Dates Covered by Report: | **10/1/91 -4/30/93** |
| Manuscript Codes (see Appendix A): | **72,130,134** |

**SUMMARY:**

**I.C.1.a** Co-investigator summary below applies.

**I.C.1.c** Co-investigator summary below applies.

**I.H** Some low boiling compounds emanating from vegetable protein hydrolysate were identified. Some indications were obtained that a mixture, which includes ammonia, of the low boiling compounds found in protein hydrolysate attracts Medflies. Ammonia added to trimedlure attracts more Medflies than trimedlure alone. Ammonia was found to attract female *Rhagoletis completa.*

**FY 93 WORK PLANS:**

**I.H** Ammonia and other low boiling compounds, and mixtures of such compounds, will be investigated for enhanced attraction of tephritids.

Investigator's Name: **F. Christian Thompson**
Affiliation and Location: **SEL, Washington, D.C.**
Research and Implementation Areas: **I.F.4**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **135**

**SUMMARY:**

**I.F.4** A prototype of the Expert System for the identification of economically important fruit flies was completed. The prototype covers some 80 species, uses some 182 characters, and includes illustrations for 43 characters and 56 species, with 10 help screens and help text. The prototype runs on MS-DOS personal computers with VGA color monitors. Continued progress was made on the biosystematic information database. The master bibliography now contains 4,486 references. The taxonomy file contains nomenclatural data for 4,192 species and 6,075 names. Host data has been accumulated and is now being entered.

**FY 93 WORK PLANS:**

**I.F.4** No significant changes have been made in objectives or approaches. All the original objectives should have been achieved by the end of 1993. A combined expert system and information database for fruit flies will be completed and available for distribution.

Investigator's Name: **J. David Warthen**
Affiliation and Location: **ICEL, Beltsville, MD**
Research and Implementation Areas: **I.B.1.a, b, c, d; I.B.2.b; I.B.5.a; I.C.2.b; I.G; I.H**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **12,14,143,144,145**

**SUMMARY:**

**I.B.1.a** QSAR of 8 racemic TML isomers was examined via computer molecular modeling (Chem-X). Correlation between Medfly catch in the field was correlated with molecular surface area, molecular volume, a torsion angle and an interatomic distance. Publication of results, July 93.

**I.B.1.b** Provided *trans*-CRL isomers/precursors for relative attractancy field studies and EAG studies. Modeled (Chem-X) the *trans*-CRL isomers and TML-C for comparison of molecular properties. No correlations are apparent so far for QSAR.

**I.B.1.c** Extracts of butterfly bush flowers were prepared for evaluation against Hawaiian fruit flies. Modeled (Chem-X) both enantiomers of alpha-copaene, but no correlations with activity are apparent for the (+) isomer and the attractive TML-C enantiomer.

**I.B.1.d** Made available HPLC expertise for the separation of Medfly parapheromones.

**I.B.2.b** Made available HPLC expertise for the separation of methyl eugenol alternatives.

**I.B.5.a** Made available HPLC expertise for the separation of synthetic analogs for the Mexican and West Indian fruit flies.

**I.C.2.b** Solvent rinses of coffee berries were biologically evaluated, but results of significance were not observed because of solvent interference.

**I.G** Provided *trans*-CRL isomers/precursors for EAG studies. Modeled (Chem-X) 8 *trans*-TML enantiomers, 4 racemic *trans*-CRL isomers and (+)-alpha-copaene, but no correlations with activity are apparent yet.

**I.H** Extracts of butterfly bush flowers were prepared for evaluation with Hawaiian fruit flies. Solvent rinses of coffee berries were biologically evaluated, but results of significance were not observed because of solvent interference. Aromadendrene was provided as a potential ovipositional attractant for Medfly.

**FY 93 WORK PLANS:**

Continue to investigate the computer molecular models of *trans*-TML enantiomers, (+) & (-)-copaene, and racemic *trans*-ceralure isomers for similar SAR. Separate the *t*-butyl esters of *cis*-ceralure isomers. Investigate exudate from red coffee berries, extract of butterfly bush flowers, and aromadendrene for Hawaiian fruit fly attractancy.

## SECTION II - EXCLUSION

### Research Summary
### Compiled by Robert L. Mangan

THE LEVELS OF EFFORT summarized in the activity reports for USDA, ARS scientists involved in Section II, Exclusion, for the period September, 1991 to October, 1992 were in nearly identical order to the approaches (A-D) given in the "Overview" of the plan. Seven of the 17 scientists reported research involving single treatment disinfestation of fruits and five reported research on fruit quality analysis from these treatments.

Successful temperature treatments were announced for a variety of tropical and subtropical fruit for the major fruit fly pests of Florida, Texas and Hawaii and for imports from Latin America and the Caribbean basin. Heat treatments for some guava varieties, mangoes and grapefruit for the Caribfly were successfully concluded. Heat treatments for mangoes, grapefruit and tangerines were completed for the Mexican fruit fly and West Indian fruit fly. Heat treatments were also completed for some stages of Medfly, melon fly and oriental fruit fly for litchi from Hawaii. Successful cold treatments were devised for canistels and white sapote fruit against the Caribfly, and for litchi and carambola against the Medfly, melon fly and oriental fruit fly.

Efforts to determine effects of temperature treatments on fruit quality included evaluations of quality of mangoes and grapefruit in several laboratories. Effects of heat treatments on beneficial organisms were evaluated for mango, grapefruit and Spondias. Other projects examined the heat transfer process in various fruit and the induction of heat shock proteins.

Very little research was carried out with fumigants. Some phytotoxicity work related to methyl bromide use was completed for blueberries (which were tolerant) and guavas (which were not). No new fumigants were identified and no new treatments were proposed for investigation using fumigants either as single treatments or as combinations or systems approaches for fruit fly disinfestation.

Fly free zone and systems approach research (B) overlaps with Control and Eradication (III) research. Population suppression approaches were not listed in this area. Other approaches included combinations of treatments to reduce oviposition such as gibberellic acid treatments for citrus against Caribfly and Mexican fruit fly. Recognition of non susceptible stages of development in certain avocado varieties were further investigated for the oriental fruit fly. Pest free periods for the walnut husk fly for peaches and nectarines were demonstrated by extensive trapping. Certain plum varieties were shown to be non-hosts for this pest. Certain lime varieties were non-hosts for the Caribfly and early developmental stages of limes were not found to be infested by Medfly, melon fly or oriental fruit fly. Basic research concerning effects of flavanoids on host status of citrus was completed for pomelos and lemons. Natural infestation levels for a variety of tropical fruits were recorded for Caribfly in Florida and relative host status for varieties of guavas and various avocado for Caribfly varieties were determined.

Specific combinations of pre- and post-harvest treatments were tested in several projects. A modified atmosphere approach using fruit coatings was tested. This treatment, in combination with heat treatments, may provide a combination treatment for some fruit. The effects of various heat treatments on quality of gibberellic acid treated grapefruit showed that gibberellic acid did not increase susceptibility to heat.

Research to derive a "total risk evaluation" (C) was carried out though most reports linked the implementation areas with more direct goals. Basic projects on host preferences, population levels, chemistry related to survival of larvae in various hosts, and the modes of action of various treatments provide data for interpretation and implementation of holistic approaches. Results of

treatments or ecological relationships that do not provide quarantine security in themselves are valuable for these approaches. As in research related to fly free zones, this approach relies heavily on other research areas, especially the basic (IV) biology area.

No projects were submitted that were classified as "Inspection/Interception" (D). Biochemical and physical approaches to finding illegal fruit or infested fruit do not appear to have received attention from ARS scientists. It should be noted, however, that host status research and identification of fly free periods described above are important components of interception programs.

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|---|---|
| Investigator's Name: | **John W. Armstrong** |
| Affiliation and Location: | **USDA, ARS, Hilo, HI** |
| Research and Implementation Areas: | **II.A.1, II.A.1.c.0.1, II.A.1.c.0.2, II.A.1.e, II.A.2.c** |
| Dates Covered by Report: | **10/1/91 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **21,28,30** |

## SUMMARY:

**II.A.1** A 12-day, 1°C treatment against Medfly, melon fly, and oriental fruit fly was developed for carambola. Litchi hot water treatment data was completed for Medfly, melon fly, and oriental fruit fly eggs and first instars. Mango forced hot-air treatment data for Medfly, melon fly, and oriental fruit fly nears completion. Rambutan were damaged by all heat and cold treatments tested. Rambutan and atemoya tests were stopped due to crop losses from Hurricane Iniki.

**II.A.1.c.0.1** Thermal mortality models for Malaysian fruit fly, Medfly, melon fly, and oriental fruit fly showed significant variation in heat tolerance between early- and late-aged eggs and larvae. Cold tolerance studies were initiated.

**II.A.1.c.0.2** California 'Marsh Ruby' and 'Marsh White' grapefruit and 'Valencia' orange, but not 'Navel' orange, tolerated forced-air treatment times and temperatures required to kill Medfly, melon fly, and oriental fruit fly eggs and larvae.

**II.A.1.e** Cooperative contact with HortResearch of New Zealand and several interested industrial research companies on the use of microwaves as a disinfestation treatment technology was maintained.

**II.A.2.c** Fruit-fly host-status studies were initiated with limes. Green to color-break limes on trees were not infested, but yellow limes on the ground were infested.

## FY 93 WORK PLANS:

**II.A.1** Complete forced hot-air mango treatment data. Continue treatment tests with atemoya and develop methods to prevent treatment injury to rambutan when fruit are available. Initiate forced hot-air treatment tests with carambola.

**II.A.1.c.0.1** Continue thermal mortality modeling of Malaysian fruit fly, Medfly, melon fly, and oriental fruit fly eggs and larvae.

**II.A.1.c.0.2** Initiate forced hot-air treatment efficacy tests with California citrus.

**II.A.1.e** Maintain contacts; no research planned.

**II.A.2.c** Continue host status studies with limes in field; initiate forced-infestation tests.

Investigator's Name: **Carrol O. Calkins**
Affiliation and Location: **IABBBRL, Gainesville, FL**
Research and Implementation Areas: **II.C.2.a**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **7,8,9,51,54,69,118,126,142**

**SUMMARY:**

**II.C.2.a** Tested equipment at Miami Airport on fruit confiscated from passengers and on fruit arriving at the Port of Miami during one week in November, 1991. Concluded that these types of sites were not appropriate for this type of detection. This type of detection is more appropriate for use on fruit at the point of embarkation.

**FY 93 WORK PLANS:**

**II.C.2.a** None.

Investigator's Name: **Harvey T. Chan, Jr.**
Affiliation and Location: **USDA, ARS, TFVRL, Hilo, HI**
Research and Implementation Areas: **II.A.1.c.0.2**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **41,42,61**

**SUMMARY:**

**II.A.1.c.0.2** Expertise and knowledge on heat transfer and thermal diffusivity have been recruited and have been actively assisting in the development of Model Systems which can predicate and describe the heating process on efficacy of insect kill, damage to fruit metabolic systems, and fruit pathogens. Preconditioning methods are being developed which alleviate chilling injury symptoms in avocados which are cold stored for the disinfestation of fruit flies as a quarantine treatment at 1°C for 14 days.

**FY 93 WORK PLANS:**

**II.A.1.c.0.2** Continue cooperative research with consulting mathematicians on development of model systems for the quarantine heat treatments of fruit. Continue development of cold storage quarantine treatments for 'Sharwil' avocados.

Investigator's Name: **Walter P. Gould**
Affiliation and Location: **USDA, ARS, SHR, Miami, FL**
Research and Implementation Areas: **II.A.1.c.0.1, II.A.1.d**
Dates Covered by Report: **9/1/92 - 4/30/93**
Manuscript Codes (see Appendix A):

**SUMMARY:**

**II.A.1.c.0.1** Hot-water immersion was tested as a quarantine treatment for guavas infested with the Caribbean fruit fly. A treatment of 35 minutes at 46.1°C killed 181,556 larvae with no survivors. Red-pulped varieties tolerated the treatment with no loss of quality. White-pulped varieties were damaged by the treatment. Currently only red-pulped guavas are commercially grown in Florida.

**II.A.1.d** Relative cold tolerance of eggs and larvae has been determined for exposed stages and insects within fruit. A manuscript is being prepared for publication. Ability of adult flies to withstand short periods of cold was determined.

**FY 93 WORK PLANS:**

**II.A.1.c.0.1** Future work will use forced hot air to treat guavas to avoid phytotoxicity problems with white-pulped varieties. Hurricane Andrew damaged guava plantings in South Florida so fruit availability may be a problem.

**II.A.1.d** Future work will correlate mortality with the supercooling point of larvae, determine if acclimation to cold or heat occurs, and determine adult thresholds of torpor and egg laying and pupal cold mortality.

Investigator's Name: **Patrick D. Greany**
Affiliation and Location: **IABBBRL, Gainesville, FL**
Research and Implementation Areas: **II.A.2.a.0.2**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **19,20,21,22,23,118**

**SUMMARY:**

**II.A.2.a.0.2** Field cage bioassays of gibberellic acid (GA) treated vs. untreated fruit were performed at Merritt Island, FL, to validate previous laboratory bioassay studies on increased resistance of GA-treated fruit to attack by *Anastrepha suspensa*. Cooperative tests were performed on use of GA to reduce susceptibility of the fruit to *Anastrepha ludens*. All field tests showed that GA-treated fruit were not only much less susceptible to fruit fly attack for an extended period, but also showed a reduced propensity to spontaneously drop from the trees late in the season.

**FY 93 WORK PLANS:**

**II.A.2.a.0.2** Tests will be conducted to evaluate the effectiveness of different formulations of GA and associated surfactants, including three commercially available surfactants in wide use. Methods to nondestructively and rapidly measure fruit peel senescence will be developed as an alternative to current methods. Economic analyses will be performed to evaluate the cost-effectiveness of GA treatments for fruit fly prophylaxis in citrus.

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| Investigator's Name: | **Guy J. Hallman** |
| Affiliation and Location: | **USDA, ARS, SHR, Miami, FL** |
| Research and Implementation Areas: | **II.A.1, II.A.1.a, II.A.1.b, II.A.1.c.0.1** |
| Dates Covered by Report: | **9/1/92 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **25,26,27,28,29,30,90,115** |

## SUMMARY:


**II.A.1** Hot water immersion, cold storage, and methyl bromide were tested on canistels and white sapotes. Storage at 1°C for up to 17 days did not damage either fruit. Neither fruit would tolerate hot water immersion or methyl bromide.

**II.A.1.a** Guavas did not tolerate methyl bromide dose sufficient to kill 99.9968% of Caribbean fruit fly immatures inside without damage to guavas. A dose close to 40 g/m$^3$ at 23°C for 2 hours would probably be needed, and guavas cannot tolerate more than 30g/m$^3$.

**II.A.1.b** The fruit coatings studied caused considerable mortality to Caribbean fruit fly immatures inside various fruits. The coatings varied in the amount of mortality reached. Hot air treatment of coated grapefruits significantly reduced treatment time. Manuscripts are in preparation.

**II.A.1.c.0.1** Caribbean fruit fly third instars reared at 30°C were more tolerant of immersion in hot water (both as 'naked' larvae and inside fruit) than those reared at 20°C. A manuscript has been accepted. Data on larval distribution depth in grapefruits has been gathered. When these data are fitted to a model, that model will be combined to probit analysis and tested for fit to experimental data on heat kill of Caribbean fruit fly inside grapefruits.


## FY 93 WORK PLANS:

**II.A.1** No future work planned for canistels or white sapote. Canistel work is being written for publication.

**II.A.1.a** Considering the possible reduction of methyl bromide use and the recent availability of other quarantine treatments for guavas (hotwater immersion and hot air), no further research is planned. However, work is being written for publication.

**II.A.1.b** More types of coatings will be tested. Effort will be made to design a coating specifically for fruit fly kill. More effort is being given in combining coatings with other treatments, such as heat, insecticide, irradiation, and fumigants.

**II.A.1.c.0.1** Current research involves testing other mediums besides water for determining heat mortality of Caribbean fruit fly immatures and to fit data on larval distribution depth to models and examine the results.

Investigator's Name: **James D. Hansen**
Affiliation and Location: **USDA, ARS, SHR, Miami, FL**
Research and Implementation Areas: **II.A.1.c.0.1**
Dates Covered by Report: **9/1/92 - 4/30/93**
Manuscript Codes (see Appendix A): **35,36,37,38,39,40,41,42,43,44, 45,49,131,132,133**

**SUMMARY:**

**II.A.1.c.0.1** Availability of infested papayas was limited because the recently planted orchard was not productive until the summer of 1992. In August, Hurricane Andrew destroyed the orchard.

**FY 93 WORK PLANS:**

**II.A.1.c.0.1** Allow orchard to recover and establish replacements for missing plants. If infested papayas become available, treat fruits with forced-air, vapor heat and hot water dips using current schedules for similar sized fruits as standards.

Investigator's Name: **Michael K. Hennessey**
Affiliation and Location: **USDA, ARS, SHR, Miami, FL**
Research and Implementation Areas: **II.A.2, II.A.2.c**
Dates Covered by Report: **2/1/92 - 4/30/93**
Manuscript Codes (see Appendix A): **56,57,58,59**

**SUMMARY:**

**II.A.2** Completed one season of research with Caribbean fruit fly in FL demonstrating natural infestation level of packing house fruits of two grades, that common guava is nonhost during early phenological stages, that the local market will buy infested fruits, and that aerial malathion does not suppress oviposition or adult female activity as determined by McPhail trapping in a commercial grove. This research complements that of W. Gould (II.A.1.c.0.1). Analyzed McPhail trap catches of Caribbean fruit flies from fifty locations county-wide and thirteen hosts over five years. Results suggested that varieties which are harvested during periods of low fly activity will have lower rates of natural infestation.

**II.A.2.c** Published results of research demonstrating nonhost status of 'Tahiti' lime to Caribbean fruit fly in FL. Bioassayed five commercial FL avocado cultivars as hosts of Caribbean fruit fly over one season and ranked them according to an index of resistance.

**FY 93 WORK PLANS:**

**II.A.2** In FL commercial common guava, pinpoint the phenological period of Caribbean fruit fly nonhost status; evaluate efficacy of fungicides in suppressing oviposition and inducing mortality.

**II.A.2.c** Standardize bioassay screening of avocado cultivars for resistance to Caribbean fruit fly infestation. Resistant varieties will be recommended to FL growers.

Investigator's Name: **Eric B. Jang**
Affiliation and Location: **TFVRL, Hilo, HI**
Research and Implementation Areas: **II.A.1.b, II.A.1.c.0.1, II.A.2**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **10,17,60,61,62,71,88**

**SUMMARY:**

**II.A.1.b** Further efficacy testing of shrinkwrap films on papaya have been delayed due to the long development time needed for proper wrapping of the fruits. University of Idaho, holder of the patent on the use of shrinkwrap films for disinfestation of insects on fresh commodities, is working with manufacturers to develop improved machinery. Initial studies with shrinkwrap on avocados did not appear to work satisfactorily.

**FY 93 WORK PLANS:**

**II.A.1.b** No further work plans on papaya are anticipated at this time. Evaluation of shrinkwrap on other fruits will be carried out as needed. Basic physiology studies of fruit flies to physical and chemical vulnerability will be initiated to support ongoing research on alternatives to methyl bromide.

**II.A.1.c.0.1** Kinetics of thermal death in fruit flies will be initiated in those species which have not yet been studied. Intermediate temperature disinfestation of fruit flies in avocados initiated last year will be continued. Studies on thermal threshold for selected species will be initiated.

**II.A.2** Specific elements of the systems approach will be carried out to validate their role in over all risk reduction for quarantine systems.

Investigator's Name: **Nicanor J. Liquido**
Affiliation and Location: **TFVRL, Hilo, HI**
Research and Implementation Areas: **II.A.2.c**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **63,72,73,74,75,76,77,78,79**

**SUMMARY:**

**II.A.2.c** Studies that aim to evaluate the integrity of the status of 'Sharwil' avocado as a "non-host" of oriental fruit fly, melon fly, and Mediterranean fruit fly are in progress.

**FY 93 WORK PLANS:**

**II.A.2.c** Investigate the factors that determine the rate of survival of oriental fruit fly eggs and larvae in 'Sharwil' avocado.

Investigator's Name: **Robert L. Mangan**
Affiliation and Location: **SARL, CQFIR, Weslaco, TX**
Research and Implementation Areas: **II.A.1.c.0.1**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **80,81,109,119**

**SUMMARY:**

**II.A.1.c.0.1** Hot forced air (HFA) treatment for grapefruit was approved by APHIS-PPQ. Data for HFA treatment of mangos completed for *Anastrepha ludens* and submitted to APHIS-PPQ. Data collection and analysis is complete for *A. ludens* on tangerines. A cooperative agreement has been developed for construction of a HFA treatment unit in Metapa, Chiapas, Mexico for treatment research for guavas and other tropical fruit. Preliminary results show that for several citrus, treatment times can be reduced using forced vapor heat during the run-up phase of heating.

**FY 93 WORK PLANS:**

**II.A.1.c.0.1** Complete summary report for tangerine treatment. Complete lab tests and confirmatory tests for Valencia oranges for *A. ludens*. Continue cooperative work with Sanidad Vegetal. Start lab tests for forced vapor heat treatments of grapefruit, tangerines, and Valencia oranges.

Investigator's Name: **Roy E. McDonald**
Affiliation and Location: **USHRL, Orlando, FL**
Research and Implementation Areas: **II.A.2.a.0.2**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **6,23,89,90,91,92,93,94,95,96,97,98, 99,100,101,102,103,118**

**SUMMARY:**

**II.A.2.a.0.2** There was no difference in market quality attributes of 'Marsh' grapefruit treated with 10 ppm gibberellic acid compared with nontreated fruit following a vapor-heat treatment. The test was run to determine if GA treatment would mitigate the injurious effects of the vapor-heat treatment. Gibberellic acid at 10 ppm was more efficacious when the surfactant Silwet L-77 was used at higher rates. At higher surfactant rates, grapefruit retained a greener color, were more resistant to puncture, and had a lower fruit fly emergence rate in bioassay tests compared with lower surfactant rates. These GA-related effects were apparent on a monthly basis from December through April.

**FY 93 WORK PLANS:**

**II.A.2.a.0.2** Because all attempts to use hot water as a potential quarantine treatment for grapefruit have failed because of resulting peel damage, GA-treated grapefruit will be used to determine if the damage can be reduced. To determine the ubiquity and reliability of the GA treatment effect, grapefruit in several representative groves throughout the state will be tested. Horticultural aspects including color, resistance of peel to puncture, fruit maturity, and taste preference will be determined on red as well as white grapefruit treated at several combinations of GA and surfactant levels.

Investigator's Name: **Raymond G. McGuire**
Affiliation and Location: **USDA, ARS, SHR, Miami, FL**
Research and Implementation Areas: **II.A.1.d.0.3, II.E.1.j.0.2**
Dates Covered by Report: **1/1/92 - 4/30/93**
Manuscript Codes (see Appendix A): **11,83,84,85**

## SUMMARY:

**II.A.1.d.0.3** The tolerance of mangoes, grapefruits, sweet potatoes, and golden apples (*Spondias cytherea*) to hot water immersion and forced hot air has been tested in research that emphasizes fruit quality after quarantine treatment. Within the tolerance range, a treatment that kills the target pest can then be selected by entomologists. Mangoes are relatively resistant to heat, and constant temperature hot water immersion (CTHWI) at 46°C for 90-115 min not only is the quickest treatment, it most effectively kills the fungus that causes the disease anthracnose. Hot air (HA) treatment to 48°C is tolerated, however, but it is longer and less effective for disease control. Grapefruits do not tolerate CTHWI at 48°C for 2 hr - the time needed for fruit fly eradication, but a gradual increase to this temperature over 3 hr is much less damaging. Hot air treatment is preferable, however, for 3 hr at 48°C. By pretreating the fruits at 27 or 32°C for 12 hr before HA treatment, damage, primarily an increased susceptibility to the fungal pathogen *Penicillium digitatum*, can be reduced. Hot air is also preferable for treating sweet potatoes; water immersions increase susceptibility to decay and aid the transmission of pathogens.

**II.E.1.j.0.2** Heat and other quarantine treatments drastically reduce surface populations of beneficial microorganisms that may antagonize pathogens with which fruit may become inoculated in storage. These populations can drop from $10^5$ colony forming units (cfu) / $cm^2$ of fruit surface to $10^1$ cfu. By reapplying a yeast to grapefruits in various citrus coatings after HA treatment, decay can be reduced. Although many coatings are toxic to the yeast, a USDA product using methylcellulose in water may actually support higher population levels.

## FY 93 WORK PLANS:

**II.A.1.d.0.3** In cooperation with entomologists, quarantine treatments of sweet potatoes, mangoes, and guavas will be developed that will minimize any loss of market quality. Although most treatments will use CTHWI or HA to eradicate insect pests, the use of controlled or modified atmospheres and fruit coatings will also be considered. Treatments will concomitantly attempt to reduce postharvest decay whenever possible.

**II.E.1.j.0.2** Where treatment increases susceptibility to disease, efforts will continue to evaluate the application of yeasts in commercial fruit coatings for biological control of postharvest pathogens. This work will continue with grapefruits; however, changes in numbers of epiphytes on mangoes will also be investigated. An attempt will be made to develop coatings that specifically promote the growth of biocontrol organisms that are antagonistic to postharvest pathogens.

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| Investigator's Name: | **William R. Miller** |
| Affiliation and Location: | **USHRL, EQI, Orlando, FL** |
| Research and Implementation Areas: | **II.A.1.c.0.2, II.A.1.d, II.A.1.f, II.A.2.a.0.2** |
| Dates Covered by Report: | **10/1/91 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **6,89,90,91,92,93,94,95,96,97,98,99, 100** |

## SUMMARY:


**II.A.1.c.0.2** No studies were conducted on mangos mainly due to Hurricane Andrew. No studies were conducted on passion fruit related to quarantine treatment mainly due to the effects of Hurricane Andrew. Some preliminary studies on the cold temperature storage related to shelf life of lychees was conducted.

**II.A.1.d** Several tests indicated that carnauba wax (2% or 6%) applied to carambolas prior to cold treatment did not reduce or eliminate chilling injury during treatment and subsequent storage.

**II.A.1.f** 'Climax' rabbiteye blueberries were found to tolerate low energy irradiation (source: electron beam) to about 1.0 kGy without serious reduction in market quality. Irradiation may prove to be an alternative to methyl bromide as a feasible treatment against quarantine pests of blueberry fruit.

**II.A.2.a.0.2** Grapefruit treated with gibberellic acid (GA) at 10 ppm tolerated vapor heat treatment (core temperature at 43.3°C for 50 min) similarly as did non GA-treated fruit, without damage to peel or loss of flavor.


## FY 93 WORK PLANS:

**II.A.1.d** Studies will be initiated on the effect of degreening on carambolas response to chilling injury when subjected to cold treatment for quarantine treatment against the Caribbean fruit fly.

**II.A.1.f** Continue experiments on the effect of low dose irradiation on blueberry market quality that may be feasible for quarantine security against the blueberry maggot, apple maggot or plum curculio.

**II.A.2.a.0.2** Studies will be initiated on the effect of low dose irradiation on GA-treated grapefruit on post harvest quality at levels to kill the Caribbean fruit fly. Experiments will be initiated on the effects of slow-run-up water temperature to the target (43.5°C) on postharvest quality of GA treated or nonGA-treated grapefruit.

Investigator's Name: **Elizabeth J. Mitcham**
Affiliation and Location: **USHRL, Orlando, FL**
Research and Implementation Areas: **II.A.1.c.0.2**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **89,92,95,101,102,103**

**SUMMARY:**

**II.A.1.c.0.2** The physiological and biochemical response of mango fruit to vapor heat treatments was explored. $CO_2$ accumulated to 13% and $O_2$ decreased to 6% in the internal atmosphere of vapor-heated mangos. Ethanol, methanol, and acetaldehyde concentrations increased, electrolyte leakage increased and ethylene-forming enzyme (EFE) activity decreased immediately after heat treatment. However, by 3 days after treatment, electrolyte leakage decreased to control levels and EFE activity recovered. Color development and loss of firmness were greater effected in inner vs. outer mesocarp tissue. Polygalacturonase (PG) activity was reduced by vapor heat treatment with longer treatment time resulting in decreased PG activity. PG activity did not recover to control levels but continued to increase with ripening. Cellulase activity was unaffected in inner mesocarp tissue, but was reduced in outer mesocarp tissue of fruit during more severe vapor heat treatments. Reduction in rate of softening indicate a reduced rate of ripening, which may be beneficial in increasing shelf life. Mango fruit have the capacity to recover from vapor heat quarantine treatment.

**FY 93 WORK PLANS:**

**II.A.1.c.0.2** None.

Investigator's Name: **Jennifer L. Sharp**
Affiliation and Location: **USDA, ARS, SHR, Miami, FL**
Research and Implementation Areas: **II.A.1.c.0.1**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **27,39,56,58,90,93,114,115,116,117**

**SUMMARY:**

**II.A.1.c.0.1** Hot air quarantine treatments were developed against Caribbean fruit fly eggs and larvae - one for grapefruit and one for mangos. Quarantine security is provided against the pest in a commodity when the center pulp of grapefruit and the seed surface in mango is ≥ 44°C.

**FY 93 WORK PLANS:**

**II.A.1.c.0.1** Data are being gathered concerning microwaves as a quarantine treatment against Caribbean fruit fly in commodities; irradiation is being investigated as a quarantine treatment against sweet potato weevil stages in sweetpotatoes and yams.

Investigator's Name: **Anthony C. Waiss**
Affiliation and Location: **WRRC, Albany, CA**
Research and Implementation Areas: **II.A.2.a.01, II.A.2.a.02**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A):

SUMMARY:

**II.A.2.a.01** Methyl transferase from orange (cv. Madam Vinus) has been purified and amino acid sequence has been initiated.

**II.A.2.a.02** Cooperative study with Eric Jang (TFVRL, Hilo, HI) and T. Williams (NCGPR, Riverdale, CA) has identified several lines of lemon and pummelo that are highly resistant to Mediterranean fruit fly throughout the growing season. The nature of the resistance is yet to be determined, but, contrary to earlier expectation, it is not correlated to flavonoid content of the fruit.

FY 93 WORK PLANS:

**II.A.2.a.01** No further studies are planned for the methyl-transferase enzyme in orange.

**II.A.2.a.02** No further studies are planned to study the nature of fruit fly resistance in lemon and pummelo unless more technician support for fruit fly bioassay is available.

Investigator's Name: **Victoria Y. Yokoyama**
Affiliation and Location: **USDA, ARS, HCRL, Fresno, CA**
Research and Implementation Areas: **II.A.1.d, II.A.2.a.0.2, II.B.3, II.C.4**
Dates Covered by Report: **7/1/91 - 4/30/93**
Manuscript Codes (see Appendix A):

SUMMARY:

**II.A.1.d** Shipments of California grown peaches and nectarines to New Zealand have been suspended since the summer of 1989 because of potential walnut husk fly, *Rhagoletis completa* Cresson, infestations in the fruit. Control tactics that are under development to provide quarantine security and to rescind the suspension on stone fruit exported to New Zealand are a methyl bromide fumigation, a pest-free period when stone fruit could be shipped without treatment and the poor host status of stone fruits.

**II.A.2.a.0.2** Peaches, nectarines, plums and walnuts were exposed to walnut husk fly oviposition in choice tests in the field and choice and no-choice tests in the laboratory. The acceptability of the fruit was determined by oviposition, pupation and adult emergence. The results showed that peaches and nectarines are less acceptable than walnuts, and plums were not a natural host. Plums can be shipped to New Zealand without the risk of husk fly infestation.

**II.B.3** A pest-free period for walnut husk fly in the San Joaquin Valley of California from the beginning of stone fruit harvest in the spring through July 1 of each year was verified by an extensive trapping program in walnuts in five counties using yellow sticky traps baited with ammonium carbonate. The pest-free period for walnut husk fly has been accepted in concept by regulatory agencies. The earliest

emergence of walnut husk fly occurred on July 11 in 1991 and on June 26 in 1992. Laboratory tests showed that the preovipositional period was approximately nine days. The obligatory preovipositional period helps support the validity of the pest-free period during years such as 1992 when adult emergence occurs at an earlier date than expected. Highest populations of walnut husk fly in walnuts were found in traps after the greatest volume of stone fruit had been harvested, lowering the risk of infestation after the pest-free period has ended.

**FY 93 WORK PLANS:**

**II.A.1.d** Work will progress to demonstrate that low temperature storage of stone fruits will control walnut husk fly. The methyl bromide fumigation developed in 1990 was accepted by regulatory agencies in 1992 with the provision that fruit will be inspected for infestation prior to export.

**II.B.3** The extensive trapping program that was initiated in 1991 to verify the pest-free period will be continued in 1993.

**II.C.4** The immunoassay procedure is available for experimentation to detect insect infestation in stone fruits. The procedure will be investigated as a packinghouse inspection tool that may enable regulatory agencies to determine potential walnut husk fly infestations in a small sample of packed fruit.

## SECTION III - CONTROL AND ERADICATION

### Research Summary
### Compiled by Eric B. Jang and David R. Lance

### Chemical Pesticides

Continuing concerns over the use of malathion in aerial applications necessitate the need to develop suitable chemical control agents for fruit flies. Alternatives to conventional insecticides are sorely needed for controlling tephritids. Several conventional pesticides were tested against fruit flies as potential alternatives to malathion. Carbaryl and permethrin were not effective against fruit flies in Hawaii, however, dibrom did show some activity. Five percent cyromazine was found to be effective against Mexfly (*A. ludens*). Microbials were not tested due to problems with Hawaii's quarantine regulations. No work was done on alternatives to current soil drenches. Future research in this area should concentrate on development of environmentally safe and publicly acceptable chemicals which do not affect non-target organisms or beneficial biocontrol species where possible.

### Male/Female Annihilation

Male annihilation technology (MAT) continues to be more environmentally benign than the use of bait sprays. The technique has been shown to be effective against oriental fruit fly and hopefully can be effective against other tephritids for which good attractants are available. Development of male and female annihilation technology is needed for many other species of fruit flies. Improved fractional crystallization techniques have been used to prepare concentrated fractions of the Medfly attractant ceralure. Polymer plug dispensers of ceralure were evaluated in the field and found to be more attractive than trimedlure after 3 to 4 months. Polyethylene panels containing trimedlure or ceralure were less attractive than conventional plastic panels containing stickem/lure. Environmental assessments have been submitted for a demonstration male annihilation test on the island of Kauai. An attracticide system similar to that tested on Caribfly was attractive to female Medflies and Mexflies in Guatemala. Several potential attractants appear promising in the field for possible MAT applications, especially against Malaysian fruit fly. Future research in this area is dependent on identification of attractants (see Section I) as well as development of practical field applications which could be used by state and federal action agencies.

### Autocidal Control (SIT)

Autocidal control may be the most environmentally acceptable technique but is also the most complex of the available eradication technologies. It demands the greatest resources and skill in application. High quality, laboratory-reared flies are essential to autocidal control programs. Methods were developed to alleviate acetic acid production and control pH in mass-rearing operations of Medfly, a problem in some mass-rearing facilities. Several natural product supplements were tested for nutritional importance in the Malaysian fruit fly. An artificial diet has been developed for *A. obliqua*, but efforts to rear *Toxotrypana curvicauda* were less successful. An agar-based diet was tested on Caribfly with variable results. Genetic fingerprint differences between lab strains of Caribfly have also been completed.

Sex-lethal gene sequences from Drosophila are being used to look for homologous DNA from *A. suspensa*. Transposable elements have been identified in a wide range of insect orders including fruit flies.

Much of the continuing research on developing new strains for autocidal control programs has centered on the possibility of rearing and releasing flies of a single sex (typically males). A pupal color sexing strain of Medfly allowing for a males only release has shown significant promise in

reducing wild fly populations in field tests on commercial coffee in Hawaii.

Large scale releases of one million sterile flies per square mile over a 25 mile area of commercial coffee on the island of Kauai over a six month period failed to suppress wild Medfly populations to date. Behavioral studies have uncovered some problems in sterile flies released from aircraft. A mechanical drop system for release of sterile Medflies from helicopter has been tested in mountainous regions of Hawaii and resulted in 22% and 58% reductions in populations on Kauai. A morphometric technique for identifying the source of sperm in spermathecae of females has been developed to evaluate efficacy of sterile Medfly releases and may be applicable to other species. Sampling systems have been tested to evaluate the effects of SIT on wild *Anastrepha* populations.

Gamma irradiation did not appear to adversely affect production of pheromonal components of Medfly in Guatemala. *A. obliqua* males required >100 Gy for 99.5% sterility in males. Preadult eyecolor was established as a marker for irradiation age of oriental fruit fly.

**Biocontrol**

The use of pathogens and parasitoids for biocontrol may be one of the most promising new techniques for fruit fly control. Like autocidal control, it demands large quantities of biocontrol agents and relies heavily on knowledge of the pathogen/parasite-host fly interactions in order to be successful. Initial augmentative releases of *D. longicaudata* in guava did not reduce oriental fruit fly populations on Kauai. Additional experiments were delayed due to Hurricane Iniki. Mass releases of *D. longicaudata* did however result in substantial reduction of Caribfly populations. Adult releases were more effective than pupal releases. *P. fletcheri* reduced populations of melon fly in mixed vegetable plots. Bait-entomopathogenic nematodes were not effective against Caribfly adults. Ecological studies suggest that *B. arisanus* may share fruit fly hosts with other parasites in residential areas of Hawaii.

Compliance: Section III contained 58 separate projects headed by a total of 25 Principal Investigators (PIs). Reports were received from 20 PIs who were collectively responsible for 51 of the projects. Forty-two projects were specifically addressed in the enclosed summaries. A majority of the summaries reported some progress in their areas. In all but a few cases, the PIs have indicated that they will be continuing the research in these areas (see FY 93 Work Plans).

Investigator's Name: **Carrol O. Calkins**
Affiliation and Location: **IABBBRL, Gainesville, FL**
Research and Implementation Areas: **III.C.2.a, III.C.2.b, III.D.2.b.0.1, III.D.2.c.0.4**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **7,8,9,51,54,69,118,126,142**

**SUMMARY:**

**III.C.2.a,b** Developed and improved the research quality control of mass reared Caribbean fruit flies in relation to development rate, size, eclosion rate, startle response, flight ability and mating behavior. Evaluated the quality of mass reared Medflies in the factories in Mexico and Guatemala.

**III.C.2.a,b** Periodicity of mating tests of mass reared Caribflies indicated that reared flies mated in afternoon. Wild flies mate near dusk.

**III.D.2.c.0.4** We developed strategies for inundative releases of parasites and sterile Caribbean fruit flies for population suppression.

**New Project:** We tested for presence of sex peptide in Caribfly males that inhibits remating by females.

**FY 93 WORK PLANS:**

**III.C.2.a,b** The interactive mating test between reared and wild Caribflies will be conducted.

**III.D.2.c.0.4 and III.D.2.b** Technologies for rearing and release of parasitoids and population sampling Caribflies and their parasitoids have been transferred to the Florida Dept. of Agriculture.

**New Project** To ascertain if colonization of the Caribbean fruit fly has resulted in the loss of their ability to produce a sex peptide, plans are to repeat the earlier experiment with field-collected flies.

Investigator's Name: **Harvey T. Chan, Jr.**
Affiliation and Location: **TFVRL, Hilo, HI**
Research and Implementation Areas: **III.C.1.a, III.C.1.a, III.C.1.a.0.2**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **41,42,61**

**SUMMARY:**

**III.C.1.a** A method for controlling the acetic acid produced during the rearing of Mediterranean fruit flies was developed. Using a combination treatment of pasteurization and antibiotics to the fruit fly rearing media, the ubiquitous acetic acid producing microorganisms were severely reduced in numbers due to pasteurization and controlled during larval development by the bacterialstatic action of the antibiotics. Controlling the acetic acid production produced several

side benefits: 1) Earlier and greater synchrony in larval development was attained. 2) Greater puparial yields were obtained from "bad" wheat samples. 3) A constant pH was maintained throughout the larval development cycle. 4) Atmospheric acetic acid levels were reduced below OSHA-PEL limits.

**III.C.1.a** Diet supplements consisting of solanaceous fruits including capsicums were added to Malaysian fruit fly rearing media. Preliminary results indicated some improvement in larval development.

**III.C.1.a.0.2** An ARS food engineer has been contacted and discussions are ensuing with regards to the potential use of extrusion processing for the use of spent fruit fly media for recycling and also as a by-product for animal feed.

**FY 93 WORK PLANS:**

**III.C.1.a** Continue to improve the cost efficiency of the controlled acetic acid diets by use of cheaper antibiotics and/or more efficient methods of heat transfer.

**III.C.1.a** No major allocation of research activities into improving Malaysian fruit fly rearing media is anticipated at this time due to other more important research priorities.

**III.C.1.a.0.2** No major allocation of research activities into recycling fruit fly media is anticipated at this time due to other more important research priorities.


Investigator's Name: **Roy T. Cunningham, John Spencer**
Affiliation and Location: **TFVRL, Hilo, HI**

Research and Implementation Areas: **III.C.4**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **1,2,13,63,64,70,76,143,144**

**SUMMARY:**


**III.C.4** Demonstration tests of aerial sterile insect releases of Medflies in commercial coffee on the island of Kauai did not show reduction in wild fly populations at a release rate of one million flies per square mile over a 25 mile areas. Operations were temporarily suspended due to Hurricane Iniki.


**FY 93 WORK PLANS:**

**III.C.4** The current tests of aerial released sterile flies will be terminated within the year. A mass trapping test using attractants over an area of 370 acres of commercial coffee in Kauai is being planned.

Investigator's Name: **Albert B. DeMilo**
Affiliation and Location: **ICEL, PSI, Beltsville, MD**
Research and Implementation Areas: **III.B.1.d.0.5**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **2,12,13,14,63,64,143,144**

**SUMMARY:**

**III.B.1.d.0.5** Determined that fractional crystallization techniques can be used on commercial ceralure samples to prepare fractions that are more concentrated in the $B_1$ and $B_2$ isomers.

**FY 93 WORK PLANS:**

**III.B.1.d.0.5** Continue experiments to find optimum conditions for this purification method as a means of obtaining research samples of ceralure that are very high in $B_1$ isomer content for evaluation in field experiments in the male annihilation technique. Fractional crystallization methods in combination with flash chromatography will be investigated as a means of isolating the $B_1$ isomer in purified form and in multigram quantity.

Investigator's Name: **Alfred M. Handler**
Affiliation and Location: **IABBBRL, Gainesville, FL**
Research and Implementation Areas: **III.C.1.c.0.1, III.C.1.d.0.2**
Dates Covered by Report: **10/1/92 - 4/30/93**
Manuscript Codes (see Appendix A): **31,32,33,34,105,106**

**SUMMARY:**

**III.C.1.c.0.1** Gene amplification techniques using polymerase chain reactions are being used with oligonucleotide primers from the RNA binding domains of the *Drosophila melanogaster Sex-lethal* gene to generate homologous DNA from *Anastrepha suspensa* genomic DNA. Amplified DNA sequences are being characterized.

**III.C.1.d.0.2** DNA sequences showing homology to the *hobo* transposable element have been identified by gene amplification from a wide-range of insect orders, and a molecular characterization has been done on sequences from *A. suspensa*, mutant strains of *Ceratitis capitata*, and *Bacterocera dorsalis*. *In vivo hobo* mobility assays have been developed which indicate that *A. Suspensa* is permissive to *hobo* mobility, and can autonomously mobilize this transposon.

Nuclear extracts from *D. melanogaster*, along with *P* transposase cDNA, stimulates *P* mobility in *A. Suspensa*, but mobility remains significantly lower than in *D. melanogaster*.

*hobo* gene vectors containing either the β-galactosidase, β-glucuronidase, or luciferase gene have been constructed and are presently being tested by somatic transformation (transient expression).

**FY 93 WORK PLANS:**

**III.C.1.c.0.1** If *Sex-lethal* homologous DNA is identified, it will be used to isolate genomic DNA clones from *A. Suspensa.* A complete molecular analysis will be made.

**III.C.1.d.0.2** *hobo*-like elements will be isolated and characterized from tephritid species. Their functionality (and ability to cross-mobilize) will be tested by *in vivo* mobility assays. Mobility testing of *hobo* will be continued in tephritid species, and germline transformation will be attempted in species supporting mobility.

The purification and testing of nuclear extract fractions is required to identify specific positive acting cofactors for *P* vector mobility. This research is currently precluded by work on the *hobo* vector system.

Selectable markers, in conjunction with PCR analysis will be tested in germline transformation experiments.

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| Investigator's Name: | **James D. Hansen** |
| Affiliation and Location: | **USDA, ARS, SHR, Miami, FL** |
| Research and Implementation Areas: | **III.C.1.b** |
| Dates Covered by Report: | **9/1/91 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **35,36,37,38,39,40,41,42,43,44,45, 49,131,132,133** |

**SUMMARY:**

**III.C.1.b** Obtaining adequate numbers of viable eggs from caged females was a problem. Diets tested were the agar-Carib fruit fly rearing media and the agar-papaya seed. There was no success with newly hatched larvae, but late instars feed on the agar-papaya seed diet. The source of eggs from caged females declined because of lack of infested material and damage due to Hurricane Andrew.

**FY 93 WORK PLANS:**

**III.C.1.b** When mated females become available to produce eggs, current diets used for fruit fly rearing and diets modified by the addition of papaya extracts and products will be evaluated.

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| Investigator's Name: | **Ernest J. Harris** |
| Affiliation and Location: | **TFVRL, Honolulu, HI** |
| Research and Implementation Areas: | **III.D.2.e.0.3** |
| Dates Covered by Report: | **10/1/91 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **46,47,48** |

**SUMMARY:**

**III.D.2.e.0.3** Initial ecological studies on *Biosteres arisanus* were focused on determination of the spatial distribution and host selection of this parasitoid in Waipahu and Ewa residential areas where *Bactrocera latifrons*, *B. dorsalis* and *B. cucurbitae* are permanent residents. In these locations, *B. arisanus* may share fruit fly hosts

with its larval parasitoid competitors, *Diachasmimorpha longicudata, Psyttalia incisi,* and *P. fletcheri.* Results from fruit samples taken at random showed that 22 *B. arisanus*, and 16 *D. longicaudata* emerged from two guava samples. Two *P. fletcheri* emerged, one each from 2 Momordica fruit samples. The results suggest a low parasitization rate in Waipahu and Ewa.

**FY 93 WORK PLANS:**

**III.D.2.e.0.3** Studies on biology and ecology limited by available resources will be continued. Studies of the effects of interspecific competition between parasitoid species will be conducted.

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| Investigator's Name: | **Robert R. Heath** |
| Affiliation and Location: | **IABBBRL, Gainesville FL** |
| Research and Implementation Areas: | **III.B.2.c, III.C.3.a** |
| Dates Covered by Report: | **10/1/91 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **3,4,15,16,51,52,53,54,55,67,68, 122,123** |

**SUMMARY:**

**III.B.2.c** Phase 1 of the initial attracticide system, which includes trap and chemical lure, has been tested with field populations of Mexflies and Medflies in Guatemala. The system is highly attractive to females of both species. This same system has been demonstrated to be attractive to feral populations of Caribfly in Florida.

**III.C.3.a** An analytical method enabling the collection and gas chromatographic analysis of delta-1-pyrroline that is released from calling males of the Medfly was developed. Using this procedure along with previously reported methods for the analyses of geranyl acetate, ethyl-(*E*)-3-octenoate, and *E,E*- α-farnesene, we compared pheromone release among fruit-reared, factory-reared fertile, and factory-reared sterile male Medflies in Guatemala. No significant differences in pheromone production were apparent from comparisons of pheromone released from fruit-reared, factory-reared fertile and factory-reared sterile male Medflies in collection made from 0600 to 1400 hours. In collections made from 1400 to 1700 hours, however, factory-reared fertile males produced significantly more of the three major terpene components (geranyl acetate, ethyl-(*E*)-3-octenoate, and *E,E*- α-farnesene), and the factory-reared sterile males produced significantly more of the three terpenes plus delta-1-pyrroline than fruit reared males. Sterile males produced a significantly higher percentage of ethyl-(*E*)-3-octenoate, based on the four component pheromone blend, during the 1000 to 1400 hour collections. Thus, the primary difference in pheromone production among the tested flies was that the fruit-reared males produced pheromone over a shorter time period during the day. Gamma radiation did not adversely affect the amount of total pheromone produced, but did affect component ratios in the pheromone blend.

**FY 93 WORK PLANS:**

**III.B.2.c** Optimization of this system will be investigated.

**III.C.3.a** Analysis of collections made at the Hilo, HI laboratory will be completed. Collection from irradiated and factory flies will be made at the Tapacula, Mexico laboratory and compared to data obtained from the Guatemala and Hawaii facilities. Additional studies on other putative pheromone components, on detailed behavioral analysis to determine effects of change in component ratios, on the effect of stress on the competitiveness of sterile flies, etc., will be investigated to delineate potential problems that should be addressed to further optimize the Medfly SIT program.

Investigator's Name: **Michael K. Hennessey**
Affiliation and Location: **USDA, ARS, SHRS, Miami, FL**
Research and Implementation Areas: **Exclusion III.C.1**
Dates Covered by Report: **2/2/92 - 4/30/93**
Manuscript Codes (see Appendix A): **56,57,58,59**

**SUMMARY:**

**III.C.1** Experiments have been completed to determine genetic fingerprint differences between lab strains of Caribbean fruit fly using nuclear DNA and PCR. A primer library has been set up for future assays, and lab crossing experiments have been completed to determine patterns of inheritance of DNA fragments.

**FY 93 WORK PLANS:**

**III.C.1** Characterize genetic fingerprints of laboratory and wild Caribbean fruit fly populations and strains in FL using PCR. Correlate fingerprints with host preference, behavior, and other biological traits.

Investigator's Name: **C.G. Jackson**
Affiliation and Location: **TFVRL, Hilo, HI**
Research and Implementation Areas: **III.D.2.e**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A):

**SUMMARY:**

**III.D.2.e** None.

**FY 93 WORK PLANS:**

**III.D.2.e** Studies will be continued on attraction to hosts and host plants and on acceptance and preference in insectary and field tests. Behavior of laboratory-reared parasitoids will be compared to wild strains. Work has begun and will continue to improve sampling.

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| Investigator's Name: | **Eric B. Jang** |
| Affiliation and Location: | **TFVRL, Hilo, HI** |
| Research and Implementation Areas: | **III.A.2.a, III.C.1.a, III.C.1.A.01** |
| Dates Covered by Report: | **10/1/91 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **10,17,60,61,62,71,88** |

## SUMMARY:


**III.A.2.a** Several novel (proteinaceous) compounds obtained from Pat Vail of the Fresno laboratory were tested as potential toxicants against adults of four species of tephritid fruit flies in Hawaii using an adult feeding assay. None of the compounds tested showed greater than 50% mortality after 96 hr post feeding for any of the species tested. Further studies of other microbials have been held up due to state restrictions on microbial importation to Hawaii.

**III.C.1.a** A new source of Canadian-grown wheat has alleviated much of the variation in mass-reared Medflies. Dietary deletion studies suggest a minor nutritional role for wheat-mill compared to sugar and yeast. Several different protein sources have been evaluated for mass-rearing, especially for the Malaysian fruit fly, *Bacterocera latifrons*. Environmental conditions for holding adult flies are important to longevity of this species.

**III.C.1.a.01** Yeast has been identified as a critical component of the fruit fly diet. Larvae fed on different yeast products did not develop as well as on the standard yeast product. Identification of the nutritional factors in yeast necessary for development is complex.


## FY 93 WORK PLANS:

**III.A.2.a** No further studies are planned with protein products. Evaluations on microbials await approval from state regulatory agents for their importation into Hawaii which is unlikely. Identification of microbials from lab flies is being initiated.

**III.C.1.a** Studies on physiochemical and nutritional factors which impact mass-reared fruit flies will continue especially with Malaysian fruit fly. Effects of temperature on pupal-adult emergence will be studied.

**III.C.1.a.0.1** Nutritional components in yeast necessary for larval development will be studied.

**III.C.2.a** (New) Studies on the physiological effects of mating on oviposition behavior will begin, comparing laboratory normal, irradiated and wild flies.

Investigator's Name: **Barbara A. Leonhardt**
Affiliation and Location: **ICEL, PSI, Beltsville, MD**
Research and Implementation Areas: **III.B.1.d.0.4**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **1,2,14,64,70**

## SUMMARY:

**III.B.1.d.0.4** Polymer plug dispensers containing 2 g of ceralure were evaluated in a field test and found to be as attractive as similar plugs with trimedlure for the first 2 months of the test. After 3 and 4 months, the ceralure plugs were more attractive than those with trimedlure.

Polyethylene panels containing trimedlure and ceralure suspended in the polymer were made in the laboratory and field tested; the panels tended to crack and about one-half the lure was lost by evaporation during casting of the panel from molten polyethylene. In addition, these panels were less attractive than plastic panels coated with stickum/lure mixtures. Corrugated cardboard panels coated with trimedlure or ceralure in a room temperature curable polymer were far more attractive. Commercially produced polymer panels containing trimedlure and ceralure have been tested. Additional formulations are being evaluated.

A new commercially produced polymeric plug dispenser for trimedlure is under investigation. Thus far, this dispenser appears to be as, or more, effective than the currently used dispenser for Medfly detection.

## FY 93 WORK PLANS:

**III.B.1.d.0.4** Initial evaluations of commercial polymeric panels impregnated with trimedlure or ceralure will be completed. Modifications of these will be tested with the objective of using these panels instead of currently used plastic panels coated with lure in stickum. Additional formulations of ceralure will be tested. Evaluation of the new polymeric plugs containing trimedlure will be completed. If this new plug continues to be highly effective, a recommendation will be made to stipulate this formulation as an acceptable alternative to the plug dispenser currently used for detection.

Investigator's Name: **James E. Lindegren**
Affiliation and Location: **HCRL, Fresno, CA**
Research and Implementation Areas: **III.D.1.d**
Dates Covered by Report: **1/1/92 - 4/30/93**
Manuscript Codes (see Appendix A):

## SUMMARY:

**III.D.1.d** Evaluated bait-entomopathogenic nematode infective juveniles (IJ) mixture for activity to Caribbean fruit fly adults. Adults responded vigorously to a mint apple jelly-nematode bait used by PRAXIS, Sam DeFazio, P.O. Box 123, Allegan, MI 49010, for carpenter ant control. However, nematodes in bait formulations were not successfully transferred to Caribbean fruit fly adults. The IJs were not apparent in adult crop as found with Medfly adults.

Cooperative research was initiated with Bill Schroeder, USDA, ARS, Orlando, FL regarding puparial susceptibility. In progress. Mortality response of *Galleria mellonella* (L.), (Fresno, CA tests) indicated that the six entomopathogenic nematodes varied in their ability to penetrate a 5 cm sand barrier. The nematodes evaluated, listed in order of host searching efficiency, were (1) *Steinernema* sp. from the lower Rio Grand Valley (LRGV), (2) *Heterorhabditus bacteriophora*, HP 88, (3) *H. bacteriophora*, Bioenterprise strain, (4) *S. carpocapsae*, Kapow, (5) All, and (6) UK strain. At ten infective juveniles per $cm^2$ exposures, (1) *Steinernema* sp. LRGV, was over 20 times more virulent than the (4) Kapow selection. The *S.* sp. LRGV nematode should be evaluated on tephritids.

**FY 93 WORK PLANS:**

**III.D.1.d** No further work with fruit flies is planned.

Investigator's Name: **Nicanor J. Liquido**
Affiliation and Location: **TFVRL, Hilo, HI**
Research and Implementation Areas: **III.B.1.c, III.B.1.d.0.1-0.4, III.D.2.e.0.3**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **63,72,73,74,75,76,77,78,79**

**SUMMARY:** None.

**FY 93 WORK PLANS:**

**III.B.1.c** Because of less than anticipated attraction of males of Malaysian fruit fly to latilure, research emphasis will shift from development of male annihilation protocol using latilure to a search for a much better male lure.

**III.B.1.d.0.1-0.4** Will continue research on formulation and dispensing of attractants for Mediterranean fruit fly, oriental fruit fly, melon fly, and Malaysian fruit fly.

**III.D.2.e.0.3** Using information from host survey research, distribution and abundance of parasitoids of Mediterranean fruit fly, oriental fruit fly, melon fly, and Malaysian fruit fly in Hawaii will be determined.

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| Investigator's Name: | **Robert L. Mangan** |
| Affiliation and Location: | **SARL, CQFIR, Weslaco, TX** |
| Research and Implementation Areas: | **III.A.2.b, III.C.4** |
| Dates Covered by Report: | **10/1/91 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **80,81,109,119** |

**SUMMARY:**

**III.A.2.b** This is considered a long range research project and is being carried out as a low priority part of the project III.C.4. Cooperative research to screen microbials have been developed with Pat Vail, USDA, ARS, Fresno (viruses), Raymond Carruthers, USDA, ARS, Weslaco (fungi), A.J. Martinez, USDA, APHIS, Mission, and Daniel Moreno, USDA, ARS, Weslaco (insecticides, IGR's) for candidate pathogens for use in control systems. Baseline population data is being collected in Nuevo Leon plots for field test areas. An oviposition trap to sample egg production and egg sterility in target populations is being field tested. Sublethal dosages of IGR and insecticide agents are being tested.

**III.C.4** Methods to directly evaluate the effects of sterile insect release are being tested. Emphasis has been placed on developing a sampling system for egg collection in wild *Anastrepha* populations. The long term goal will be to determine the relationship between absolute sterile insect density (rather than over-flooding ratio) and rate of egg sterility. Analysis of fruit infestation data from a field test in Baja California Sur (in cooperation with Robert Wharton, Texas A&M) has shown that clustering of eggs in fruit may be reduced in populations under heavy sterile insect challenge. Field tests in replicated plots with matched controls are needed to evaluate alternative hypotheses related to density and distribution of target populations and their susceptibility to eradication by SIT.

**FY 93 WORK PLANS:**

**III.A.2.b** If promising pathogens or compounds are identified, caged tree field tests and field plots in Nuevo Leon will be initiated. Behavioral studies of exudate feeding will be continued. Field plots will be monitored as part of this project and III.C.4.

**III.C.4** Monitoring of populations in plots in Nuevo Leon will continue. Analysis of spring 1993 mangos from Baja California field test will continue for SIT release area and control. A new project with Jorge Leyva (Colegio Post-Graduados, Chapingo, MX) is being developed to further examine the effects of SIT and, also, of parasite releases on distribution of eggs and larvae in infested fruit. This will have direct effects on risk assessment for fly free zones maintained by SIT.

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| Investigator's Name: | **Donald O. McInnis** |
| Affiliation and Location: | **TFVRL, Honolulu, HI** |
| Research and Implementation Areas: | **III.C.1.c.0.1, III.C.1.c.0.3, III.C.2.b.0.2, III.C.3.d** |
| Dates Covered by Report: | **10/1/91 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **50,86,87** |

## SUMMARY:


**III.C.1.c.0.1** Field tests continue to evaluate the hybrid pupal color sexing strain Kauai, HI. The 'males-only' strain continues to perform well. Work began in a collaborative effort with the IAEA (Vienna) and MOSCAMED (Guatemala) to evaluate the new temperature sensitive (tsl) sexing strain for Medflies. Further collaborative work with the Univ. of Hawaii has yielded several promising Medfly strains through molecular approaches which may lead to improved genetic sexing systems.

**III.C.1.c.0.3** Collaborative work with USDA, ARS and E. Jang (ARS, Hilo) has focused on the behavioral (esp. mating) quality of mass-reared Medflies used in the ongoing SIT program in Kauai. Field cage mating tests have uncovered serious mating deficiencies of aircraft released, sterile flies in comparison with sterile flies not air-released. Molecular methods (PCR) have been applied to monitor the genetic quality of lab-reared vs. wild flies in Hawaii and California.

**III.C.2.b.0.2** The new technique of distinguishing sperm of irradiated and normal flies was applied in evaluating the performance of the 'males-only' genetic sexing strain in Kauai. It was also used to monitor the field mating of standard Medflies in the Kauai SIT program.

**III.C.3.d** Intensive and expanded field evaluation of the pupal color Medfly genetic sexing strain continued in Kauai coffee fields. Sterile releases commenced in April and continue to date with 2 air drops per week (except for a 6 week break in September and October due to Hurricane Iniki). The males-only strain again is achieving higher sterility in the field than the standard strain.


## FY 93 WORK PLANS:

**III.C.1.c.0.1** Research will continue to evaluate the existing pupal color strain in Hawaii; a new, more efficient strain in Guatemala (IAEA tsl strain), and possible improvements based on genetically transformed Medflies.

**III.C.1.c.0.3** Small-scale field and lab tests will continue to focus on resolving the reasons for poor field performance of sterile, released Medflies in Kauai.

**III.C.2.b.0.2** Plans include efforts to apply the technique of sperm ID to other Medfly SIT programs, and also to other tephritid fruit fly species (e.g. *Bactrocera* and *Anastrepha*).

**III.C.3.d** Field research with the genetic sexing strain will continue in Kauai hopefully until a clear result is obtained in comparing the release of males-only vs. the standard, bisexual strain. Collaborative research with the IAEA will lead to lab and field tests in Guatemala (possibly elsewhere later) with the egg-based, temperature sensitive lethal sexing strain.

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| Investigator's Name: | **Daniel S. Moreno** |
| Affiliation and Location: | **SARL, Weslaco, TX** |
| Research and Implementation Areas: | **III.A.2.b, III.C.1.b.0.1, III.C.3.a, III.C.3.b** |
| Dates Covered by Report: | **10/1/91 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **81,109** |

**SUMMARY:**

**III.A.2.b** We found a 5% cyromazine to cause high mortality of *A. ludens*. Females that did not die were unable to lay eggs on fruits having resistance to penetration >15 Newtons. The $F_1$ of treated females developed significantly less in an artificial larval diet in the laboratory and in grapefruit in the field than control insects.

**III.C.1.b.0.1** We are now able to rear *A. obliqua* on an artificial diet of corn flour, torula yeast and fresh papaya and in a diet of corn flour, torula yeast and casein. One colony has been in the former diet for 24 generations and another colony in the latter diet for 12. The addition of vitamins does not appear to be required in the diets but minerals are. A basal diet of corn flour and torula yeast that includes soya flour or pea flour appears promising.

**III.C.3.a** No work was done with the irradiation of *A. ludens*. However, we irradiated pupae of *A. obliqua* two days before emergence, and found that we could completely sterilize females with <20 Gy but could only sterilize males 99.5% with 100 Gy.

**III.C.3.b** This study was postponed to concentrate more time on the rearing of *A. obliqua*.

**FY 93 WORK PLANS:**

**III.A.2.b** We will continue to investigate potential and experimental IGR-type components that may sterilize or modify the behavior of adult *A. ludens*.

**III.C.1.b.0.1** We will evaluate the quality of soya bean flour and pea flour diets as compared and compare the results to our standard casein diet in the production of *A. obliqua*. The comparisons will run for six generations. We will also define the life cycle of *A. obliqua* at various constant temperatures to develop a day-degree developmental model.

**III.C.3.a** We hope to finish the irradiation study that we initiated with *A. obliqua*. If time permits, we will start the proposed study with *A. ludens*.

**III.C.3.b** We hope to start the proposed study the latter part of 93.

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| Investigator's Name: | **Mary Purcell** |
| Affiliation and Location: | **TFVRL, Kapaa, HI** |
| Research and Implementation Areas: | **III.A.1, III.D.2.c** |
| Dates Covered by Report: | **10/1/92 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **128,137** |

## SUMMARY:


**III.A.1** Conventional insecticides were tested in protein baits in the laboratory and LC 50's were determined. Insecticides tested were carbaryl, permethrin, dibrom and malathion. Carbaryl and permethrin were ineffective, and dibrom and malathion were the most toxic to oriental, Medfly and melon flies. *D. longicaudata* were as susceptible as the fruit flies to malathion. Due to loss of one employee that did this work, we did not test azadirachtin or cyromazine.

**III.D.2.c** Augmentative release studies of *Diachasmimorpha longicaudata* have been underway since March 1992 in a commercial guava orchard in Kilauea and a mixed strawberry guava forest in Kealia, Kauai. Reductions of oriental fruit flies were not observed either in methyleugenol traps or in the fruit collections in 1992. This is mainly because parasites could not be released earlier in the year as previously planned. However, in the Kealia release site, *D. longicaudata* began to increase in abundance and accounted for up to 80% of the parasites recovered from several fruit samples in August, 1992. Experiments were halted after Sept. 11, 1992, due to Hurricane Iniki. Releases were resumed in January 1993 in the guava orchard and in February 1993 in Kealia. We have done extensive sampling studies in Kilauea and Hilo on the effect ripening of fruit on the ground on abundance of *D. longicaudata.* The results showed that the oldest, ripest fruit contained significantly (up to 33 times higher) more *D. longicaudata*, and that previous fruit samples (in 1991-92) may have under-represented this parasite.

Augmentative release of *Psytallia fletcheri* in a mixed vegetable plot at the Wailua experiment station were made in May-August 1992. Significantly higher numbers of melon fly in cue-lure traps were observed in the control plot than in the release field. We did not find differences in larval numbers in the tomato, zucchini or cucumber samples. This may have been due to an inadequate population of melon fly, because a large percentage of the samples were uninfested.


## FY 93 WORK PLANS:

**III.A.1** Bioassays and field tests with azadirachtin and cyromazine will be initiated as soon as a technician can be hired to do this work. We do not intend to include nematodes in these trials at this time.

**III.D.2.c** Augmentative releases of *D. longicaudata* will continue through 1993. We have modified our sampling plan to more accurately estimate this parasite. We are also studying the effectiveness of yellow spheres for monitoring the distribution and dispersal of *D. longicaudata.* We plan to continue studies with *P. fletcheri* in experimental research plots in Hilo if University plots are available. The fields will be "seeded" with melon flies early in the trials to ensure sufficient infestation of the plants.

Investigator's Name: **Stanley T. Seo**
Affiliation and Location: **TFVRL, Honolulu, HI**
Research and Implementation Areas: **III.C.2.a**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A):

**SUMMARY:**

**III.C.2.a** Laboratory-reared Mediterranean fruit fly required doses of 13.5, 92.2, 136.7, 161.3 and 191.4 Gray for 50, 95, 99.5, 99.9 and 99.99 percent sterility of sperm when the pupae were gamma-irradiated in respired air. Normal lab-reared males, 150 Gy-irradiated lab-reared males and normal wild-males were similar by transferring 1175, 962 and 822 sperm to normal lab-reared females in single matings of 118, 86 and 109 minutes, respectively, but the mating period was shorter for irradiated flies. There were nontransfers of sperm during matings of 48, 47 and 74 minutes by 23, 28 and 22 percent of normal , irradiated and wild flies, respectively. Lab-reared females and wild-females were similar by accepting 1175 and 948 sperm in 48 and 72 minutes. Mating of wild-flies and cross-mating of irradiated and wild flies decreased rapidly with increase of irradiated fly to wild fly ratio in a mating cage with surface area of 6060 $cm^2$. Multiple mating by irradiated lab-reared males may improve the effect of cross-mating because the irradiated males mated 2 - 22 times in 41 days with mean numbers of 200 - 2529 sperm transferred to lab-reared females during 47 - 134 minutes or total numbers of 200 - 17,700 sperm in 41 days. Preadult eye color data was developed for oriental fruit fly to determine the optimum age for sterilization.

**FY 93 WORK PLANS:**

**III.C.2.a** In 1993, we intend to complete data on multiple mating habits of the irradiated Medfly males, durations in copula and sperm transfer for each time the male mates to permit use of simpler models that are based on surface area for correlation between field and lab data and evaluation of a sterile-insect release program and establish time intervals for the evaluation and for releasing sterile flies. Work will start on developing data on irradiation dose-sterility relationship, optimum mixed population density and optimum irradiated fly:wild fly ratio for the oriental fruit fly, melon fly and Malaysian fruit fly, including additional work with Medfly on irradiated fly:wild fly ratio.

Investigator's Name: **John M. Sivinski**
Affiliation and Location: **IABBBRL, Gainesville, FL**
Research and Implementation Areas: **III.D.2.a, III.D.2.c.0.4**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **5,51,54,55,66,121,122,123,124, 125,126**

**SUMMARY:**

**III.D.2.a** Funds have been obtained to begin a simultaneous study of *Anastrepha* species parasite ecology and behavior in Florida and tropical Mexico. The ultimate goal is to identify Mexican species for introduction into the USA and to locate those areas in Florida most suited to the new biocontrol agents. In order to discover

which natural enemies would be the most suitable additions to Florida's fauna, fruit is being sampled from different microhabitats in various hosts in both Florida and Mexico. Parasitism in these microhabitats is being correlated to light, temperature and humidity measured by computerized sensors in the field. Should our present natural enemies not forage effectively in certain microhabitats, it is hoped that a Mexican species can be imported to fill the gap. Several thousands of fruit samples had been obtained prior to the end of April.

**III.D.2.c.0.4** Mass releases of the braconid parasite *Diachasmimorpha longicaudata* (150,000/sq. mi./week) resulted in substantial suppression of Caribbean fruit fly populations (5 - 10% of control areas). The release of adults 1-5 days of age that had been fed and exposed to mates had more effect than pupae left in the field to eclose.

**FY 93 WORK PLANS:**

**III.D.2.a** With the aid of Mexican collaborators, explorations for parasites will begin during late summer in several sites in southern Mexico. Mexican parasite species will be cultured and shipment to Florida quarantine centers considered. Parasites will be tested for ability to develop on Caribbean fruit flies.

**III.D.2.c.0.4** The Florida Division of Plant Industry will continue to develop augmented parasite release techniques. Releases over a 57 square mile area to provide a buffer between suburban sites with high fly densities and citrus growing regions in the Florida Indian River area are planned for late summer. Advice will be given on experiments to discover optimal release rates and mechanisms.

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| Investigator's Name: | **Patrick V. Vail** |
| Affiliation and Location: | **HCRL, Fresno, CA** |
| Research and Implementation Areas: | **III.A.2.a** |
| Dates Covered by Report: | **10/1/91 - 4/30/93** |
| Manuscript Codes (see Appendix A): | |

**SUMMARY:**

**III.A.2.a** A number of viral isolates from throughout the world have been collected and are in storage at the Fresno location. We have been unable to conduct research on these pathogens because of problems encountered with Hawaii quarantine regulations.

**FY 93 WORK PLANS:**

**III.A.2.a** Possibly use another host such as Mexican fruit fly.

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| Investigator's Name: | **Roger I. Vargas** |
| Affiliation and Location: | **TFVRL, Honolulu, HI** |
| Research and Implementation Areas: | **III.B.1.a, III.C.2.b, III.C.3.d** |
| Dates Covered by Report: | **10/1/91 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **24,107,127,128,136,137,138,139, 140,141** |

## SUMMARY:


**III.B.1.a** An environmental assessment has been prepared and submitted for a demonstration project at Moloaa, Kauai. The pilot test will evaluate three melon fly and oriental fly male annihilation formulations: 1) combination methyl eugenol/cue-lure (+naled) traps on stakes, 2) methyl eugenol (+naled) and cue-lure (+naled) on wooden discs hung in vegetation, and 3) methyl eugenol (+naled) and cure-lure (+naled) mixed with Min-U-Gel and squirted as spots on tree trunks. The primary objectives will be to study the effects of male annihilation treatments on papaya infestation, quarantine security, and the environment.

**III.C.2.b** A 2-yr program of sterile Medfly releases was conducted on Kauai, Hawaii in commercial coffee. Mean numbers of Medflies in treated coffee fields were 22% and 58% less than in control fields during 1990 and 1991, respectively. We documented the effects of these sterile releases on a cohabitating fruit fly species, oriental fruit fly, and on an egg-larval parasitoid wasp that attacks both fruit fly species. Data are being analyzed and applied to development of Integrated Pest Management Systems (IPM) for fruit flies in agro-ecosystems.

**III.C.3.d** A mechanical drop system for release of free-falling sterile Medflies from a helicopter was developed. Subsequently, sterile fly releases by helicopter were initiated over a 15 $km^2$ area in two rugged remote valleys (Olokele Canyon and Kahana Valley) to demonstrate eradication of Medfly population in a typical forested area. Thus far, the helicopter has proved unequalled in survey and fly releases throughout this difficult terrain.


## FY 93 WORK PLANS:

**III.B.1.a** Secure necessary permits (Environmental Use Permits) and begin a test.

**III.C.2.b** Complete analysis of data and develop quality control tests.

**III.C.3.d** Continue eradication tests in Olokele and Kahana areas.

## SECTION IV - FUNDAMENTAL BIOLOGY

### Research Summary
### Compiled by Eric B. Jang and David R. Lance

### Ecology and Behavior

Ecological and behavioral studies are important in identifying characteristics which may be useful in improved methods for fruit fly control. Demographic studies of Medfly and Malaysian fruit fly as well as Caribfly have been determined. Malaysian fruit fly (*B. latifrons*) is the most recent pest tephritid to become established in Hawaii. An intensive census of Malaysian fruit fly populations in Hawaii identified six new host records for this species. A comprehensive list of host plants and natural enemies of Malaysian fruit fly was prepared. Data on infestation rates on various fruits has also been done. Stratified random sampling techniques were developed in Hawaii for monitoring of three fruit fly species. Bioassays have been developed to quantify acoustical courtship signals in Caribfly. A general methodology has been worked out to determine adult Mexfly numbers based on eggs laid in fruit. Population age structure indices have been developed to study the relationship between single and multiple populations and available host-fruit. A fluorescent analysis technique was developed to identify age of *Anastrepha* fruit flies. Mark and recapture techniques were used to estimate male population of oriental fruit flies in guava orchards. Fruit collection for larval population estimates revealed lower numbers than for adults. The complexities of male pheromone in Medflies revealed that components of the pheromone and host fruit odors can influence female behavior of this species.

### Physiology, Biochemistry and Genetics

Research in these areas promises to unlock exciting new discoveries which may be useful in control of fruit flies. Basic studies on fruit fly physiology, biochemistry and genetics are likely to reveal useful information for each of the previously described sections. Genetic studies on *A. suspensa* yolk proteins have been carried out and identified in terms of developmental, tissue and sex specificity. Certain compounds have been found to antagonize key hormone systems in Medflies. Studies on temperature tolerance and protein synthesis has shown possible thermotolerance in cultured fruit fly cells. Future studies in this area are warranted.

Compliance: Section IV of the original Action Plan contained 20 separate projects headed by 13 Principal Investigators (PIs). Summary reports were received by 12 PIs who were responsible for 18 of the projects. Sixteen projects were addressed in the reports. The PIs reported some work in 15 project areas. One additional project was reported by a PI who was not originally listed in Section IV. The majority of the PIs in Section IV also have responsibilities in other sections. Most have indicated that research will continue in most areas (see FY 93 Work Plans).

Investigator's Name: **Bruce C. Campbell**
Affiliation and Location: **WRRC, Albany, CA**
Research and Implementation Areas: **IV.B.6**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A):

**SUMMARY:**

**IV.B.6** Research for male Medfly accessory gland hormones which suppress female receptivity to mating has been discontinued.

**FY 93 WORK PLANS:**

**IV.B.6** None.

Investigator's Name: **Alfred M. Handler**
Affiliation and Location: **IABBBRL, Gainesville, FL**
Research and Implementation Areas: **IV.B.1.a**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **31,32,33,34,105,106**

**SUMMARY:**

**IV.B.1.a** The 48 kDa yolk peptide from *Anastrepha suspensa* has been identified and characterized in terms of developmental, tissue, and sex specificity. Gene expression and protein synthesis are not under any apparent control by juvenile hormone or 20-hydroxyecdysone. A YP cDNA clone has been isolated and has been used to identify three putative genomic clones.

**FY 93 WORK PLANS:**

**IV.B.1.a** The genomic clones will be restriction mapped and sequenced. DNA and amino acid sequence GenBank comparisons will be made with attention to putative regulatory elements.

Investigator's Name: **Ernest J. Harris**
Affiliation and Location: **TFVRL, Honolulu, HI**
Research and Implementation Areas: **IV.A.1.a, IV.A.1.b, IV.A.7**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **46,47,48**

**SUMMARY:**

**IV.A.1.a** Studies of Stratified random sampling in Waipahu, Oahu using male lure and liquid bait traps and fruit collections simultaneously, showed that during 1992 the mean catch/trap/day was 180.7, 49.7, and 0.02 for *B. dorsalis, B. cucurbitae,* and *C. capitata.* The population trends indicated by trap data showed *B. dorsalis* reached a peak in July and the lowest level in September. The highest number was caught in stratum 20 and the lowest number was caught in stratum 2. Peak *B. cucurbitae* trends occurred in July and the lowest

in September. The highest number was caught in stratum 22 and the lowest number was caught in stratum 12. *C. capitata* trap catches were sparse, only 6 were caught during the 12 months the traps were in operation.

The results showed that *B. latifrons* was reared for the first time from cherry tomato in Kekaha, Kauai. Subsequent findings of this insect in egg plant in Kekaha, Waimea, and Hanapepe confirms that *B. latifrons* is completely distributed throughout the Hawaiian Island chain. Another first on the island of Kauai was the rearing of 2 oriental fruit flies from kikania, *S. capsicoides*. Details about distribution and host selection was obtained from periodic collections of fruits and vegetables. *B. latifrons* was found in seven of the fifteen community gardens in Honolulu County. The largest numbers were found in Waipahu, Ala Wai, and Wahiawa community gardens. No *B. latifrons* were caught in male lure or liquid lure traps placed in all gardens. The fruit selected by the fly included purple egg plant, Laotian egg plant, hot pepper, tomatoes, and pumpkin with mean infestation rates of 8.37, 133.7, 73.2, 11.5, and 21.3 flies per Kg of fruit, respectively.

**FY 93 WORK PLANS:**

**IV.A.1.a** No studies are planned because insufficient resources are available to conduct these studies.

**IV.A.1.a** **IV.A.1.b, and IV.A.7** No further studies are planned for survey of the Malaysian fruit fly on the island of Oahu. Further studies will be conducted on the island of Kauai to determine the effects of Typhoon Iniki on the distribution and abundance of *B. latifrons*.

**IV.A.1.b** **and IV.A.7** Further studies will be limited to what is necessary to fill in gaps identified by evaluation of data collected during 1992.

Investigator's Name: **G.C. Jackson**
Affiliation and Location: **TFVRL, Hilo, HI**
Research and Implementation Areas: **IV.A.2**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A):

**SUMMARY:**

**IV.A.2** None.

**FY 93 WORK PLANS:**

**IV.A.2** The mating behavior of the Malaysian fruit fly will be studied in laboratory and outdoor screen cages. Field observations will be made on the reproductive behavior of wild and sterile Medflies.

Investigator's Name: **Eric B. Jang**
Affiliation and Location: **TFVRL, Hilo, HI**
Research and Implementation Areas: **IV.B.2.a, IV.B.5.b**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **10,17,60,61,62,71,88**

**SUMMARY:**

**IV.B.2.a** *In vitro* studies on the effects of anti-JH compounds on JH biosynthesis and release from Medfly corpora allata showed that both physiological activities were affected.

**IV.B.5.b** Heat shock proteins in cultured cell lines from the oriental fruit fly showed some similar $M_r$ proteins as those reported in Medfly cells. This work is continuing.

**FY 93 WORK PLANS:**

**IV.B.2.a** HPLC analysis of biosynthesized and released products will be carried out to establish the biochemical mode of action of the anti-JH compounds. Responses of corpora allata from other species will be initiated if time permits.

**IV.B.5.b** The effects of host plants, quarantine treatments and environmental changes on specific enzyme systems will be looked at for evidence of physiological vulnerability which could be exploited for control or improvement of mass-reared fruit flies for SIT.

Investigator's Name: **Peter J. Landolt**
Affiliation and Location: **IABBBRL, Gainesville, FL**
Research and Implementation Areas: **IV.A.4**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **51,54,55,65,66,67,68**

**SUMMARY:**

**IV.A.4** Patterns of feeding by *Anastrepha suspensa* on sucrose and yeast were documented as a function of time of day and fly age, and correlated to fly reproductive development and sexual activity.

**FY 93 WORK PLANS:**

**IV.A.4** None.

Investigator's Name: **Douglas M. Light**
Affiliation and Location: **WRRC, PWA, Albany, CA**
Research and Implementation Areas: **IV.A.8**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **17,62,71,134**

**SUMMARY:**

**IV.A.8** Studies on the identification of the Medfly pheromone have focused on both chemical reanalysis and the interaction/synergism of various volatiles with the natural, pheromonal emission of calling male Medflies. The pheromonal emission of male Medflies was reinvestigated to determine the effects of time of calling and age upon the chemical composition. In laboratory bioassays, identified intermediate and minor components synergized the attractancy of the natural male odor. Similarly, green leaf volatiles had a significant synergistic effect on capture of lab-reared virgin female Medflies in multiple-choice preference tests. Volatile components from several host fruits were found to synergize and inhibit normal fruit foraging behaviors, including attraction and oviposition, in Medflies.

**FY 93 WORK PLANS:**

**IV.A.8** Continuation of behavioral bioassays to resolve the putative pheromonal agents and host plant synergists for Medflies. Attempt such bioassays under field conditions. Continuation of plant volatile bioassays on Medflies, and expansion to *Bactrocera* species.

Investigator's Name: **Nicanor J. Liquido**
Affiliation and Location: **TFVRL, Hilo, HI**
Research and Implementation Areas: **IV.A.1.a, IV.A.2, IV.A.7.a, IV.A.7.b**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **63,72,73,74,75,76,77,78,79**

**SUMMARY:**

**IV.A.1.a** An intensive census of Malaysian fruit fly populations was conducted on the islands of Hawaii, Kauai, Lanai, Maui, Molokai, and Oahu. Sixteen cucurbitaceous and solanaceous host plants were identified; six of which were new host records. The distribution and abundance of Malaysian fruit fly were determined. Comprehensive lists of host plants and natural enemies of Malaysian fruit fly were prepared.

**FY 93 WORK PLANS:**

**IV.A.1.a and IV.A.7.a** No further work on general survey of Malaysian fruit fly in Hawaii is planned. Initiate dispersal studies on males of oriental fruit fly, melon fly, and Malaysian fruit fly. Initiate dispersal studies on females of Mediterranean fruit fly.

**IV.A.2** Initiate mating behavior studies on Malaysian fruit fly.

**IV.A.7.B** Dispersion and sampling plans for estimating densities of Mediterranean fruit fly, oriental fruit fly, melon fly, and Malaysian fruit fly in several selected host commodities will be developed.

Investigator's Name: **Robert L. Mangan**
Affiliation and Location: **SARL, CQFIR, Weslaco, TX**
Research and Implementation Areas: **IV.A.3.a**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **80,81,109,119**

**SUMMARY:**

**IV.A.3.a** The population age structure of fruit flies can be used to determine whether flies using different hosts are part of a single population that switches among hosts or different populations. This will help determine whether destruction of native hosts of *A. ludens*, such as yellow chapote, will help reduce the infestation rate in commercial citrus. We (Nada T. Carruthers and I) have adapted the fluorescence analysis technique to several species of *Anastrepha* and have shown that adult males and females can be classified in age groups with sufficient accuracy to determine age structure of populations. We have also identified the compounds that change during aging and are responsible for fluorescence changes.

**FY 93 WORK PLANS:**

**IV.A.3.a** Two projects are planned. We will expand the data base of species to include *A. striata* and other species if they are available for identification of pigments and changes with age. We will also do a field cage test with *A. ludens* to determine the best way to take samples and perform analysis.

Investigator's Name: **Richard W. Mankin**
Affiliation and Location: **IABBBRL, Gainesville, FL**
Research and Implementation Areas: **IV.A.2**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **82**

**SUMMARY:**

**IV.A.2** Acoustical technology and computer software were developed to digitize and analyze fruit fly calling songs. Songs have been analyzed from *Anastrepha suspensa, A. fracterculus, A. obliqua, A. sororcula,* and *A. grandis*. Preliminary analysis indicates that these species can be distinguished by their different pulse-train durations and inter-train intervals.

**FY 93 WORK PLANS:**

**IV.A.2** Inter- and intra-specific comparisons of calling songs are continuing. Questions will be addressed concerning the contribution of changes in calling songs on *Anastrepha* speciation.

Investigator's Name: **Daniel S. Moreno**
Affiliation and Location: **SARL, Weslaco, TX**
Research and Implementation Areas: **IV.A.3.a**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **81,109**

**SUMMARY:**

**IV.A.3.a** We have worked out the general methodology of infestation for various fruits to determine the mean number of eggs laid per fruit. From this number we can estimate proportional development of flies to the adult stage per fruit. We have developed a 1% protein solution diet that also contains 6% sugar and trace minerals and vitamins to feed preovipositing flies. The quantities of protein and sugar are based on those found in ripe grapefruit and oranges.

**FY 93 WORK PLANS:**

**IV.A.3.a** We are continuing this study and now are prepared to infest fresh fruit as it ripens on the tree. We will be starting with 'Rio Red' grapefruit.

Investigator's Name: **Mary Purcell**
Affiliation and Location: **TFVRL, Kapaa, HI**
Research and Implementation Areas: **IV.E.7.b**
Dates Covered by Report: **10/1/91 - 4/30/93**
Manuscript Codes (see Appendix A): **128,137**

**SUMMARY:**

**IV.E.7.b** Mark and recapture studies were conducted in September 1991 and in February 1992 to estimate the absolute populations of oriental fruit fly in a Kilauea guava orchard. Use of fluorescent powders to dye pupae and recapturing dyed adult males in methyl eugenol was very effective for estimating populations. In addition, absolute larval populations were estimated by counting fruits and larvae in samples of infested fruit. The larval estimates were significantly lower than estimates of adult males in the orchard.

**FY 93 WORK PLANS:**

**IV.E.7.b** No further studies are planned at this time.

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| Investigator's Name: | **John M. Sivinski** |
| Affiliation and Location: | **IABBBRL, Gainesville, FL** |
| Research and Implementation Areas: | **IV.A.2, IV.A.6.b** |
| Dates Covered by Report: | **10/1/91 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **5,51,54,55,66,121,122,123,124,125, 126** |

**SUMMARY:**

**IV.A.2** A new bioassay to quantify the response of Caribbean fruit flies to acoustic courtship signals has been developed. The role of developmental asymmetries on acoustic signals and attractiveness to females are underway. Males have been demonstrated to deposit pheromones on leaf-territory surfaces and this may influence competition between males for signaling sites.

**IV.A.6.b** Life spans and fecundities of wild and laboratory Caribbean fruit fly strains have been determined. No evidence has been found for nutrients that influence life span or fecundity being passed from males to mates via ejaculates. The presence of flies of the opposite sex significantly lowers life span in the laboratory. Data on infestation rates of various fruit over the period of a year has been obtained from locations across the Caribbean fruit fly's range.

**FY 93 WORK PLANS:**

**IV.A.2** Experiments on acoustic courtship signals will continue. The role of pheromones and signalling sites in male-male aggression will be investigated.

**IV.A.6.b** The effect of density on mortality under laboratory conditions will be determined. These studies will have a substantial impact on models of fly population dynamics and theories of senescence.

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| Investigator's Name: | **Roger I. Vargas** |
| Affiliation and Location: | **TFVRL, Honolulu, HI** |
| Research and Implementation Areas: | **IV.A.6.a, IV.A.7** |
| Dates Covered by Report: | **10/1/91 - 4/30/93** |
| Manuscript Codes (see Appendix A): | **24,107,127,128,136,137,138,139, 140,141** |

**SUMMARY:**

**IV.A.6.a** Developmental and demographic studies were completed on laboratory strains of melon fly, oriental fruit fly, Mediterranean fruit fly, and Malaysian fruit fly reared at four constant temperatures. The objective is to develop data for geographical range projections, improved rearing procedures, and developmental models during colonization of new areas.

**IV.A.7** A 2-yr program of sampling (traps and coffee fruits) Medfly in commercial coffee fields was completed. The objective is to develop non-trimedlure detecting systems.

**FY 93 WORK PLANS:**

**IV.A.6.a** Developmental and demographic studies are being conducted at fluctuating temperatures with wild strains of melon fly, oriental fruit fly, Mediterranean fruit fly, and Malaysian fruit fly reared on a natural host. When experiments are completed, appropriate GIS maps will be developed.

**IV.A.7** A program of sampling Medfly in wild areas is presently being conducted. When experiments are completed, appropriate GIS maps will be developed.

# APHIS REPORTS

## Introduction

**Compiled by Norman C. Leppla, Danny B. Gates, and Michael B. Stefan**

The Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), Methods Development (MD) fruit fly projects are conducted under the direction of the Mission Methods Development Center at Moore Air Base (Edinburg), Texas and implemented at that location, Waimanalo, Hawaii; Guatemala City, Guatemala; Gainesville, Florida; and affiliated sites throughout the world. These Methods Development Stations are operated primarily to support PPQ and APHIS International Services programs with the following two objectives:

1. To respond to immediate operational needs for scientific and technical support. This response can be in the form of information, recommendations, tests, ideas, and so forth provided at any location where help is needed or requested. Methods Development scientists must be capable, available, and effective in responding in their assigned areas of expertise.

2. To facilitate, coordinate, and adapt research for use in operational programs. New pest and disease control technologies are developed by first clearly defining the problems and then addressing them within a range of work that is only as wide as required to solve them. This work is limited to budgeted objectives and minor deviations from approved workplans. Promising directions for new work are communicated to the research community or developed into new Methods Development objectives.

Methods Development projects are reviewed by APHIS scientists, staff, and cooperators, and updated annually by means of formal objectives. Fruit fly objectives are organized by investigator, location, and subject species (see outlines). The following four broad categories and associated project titles define objectives for the field programs, regulatory programs; regulatory programs are described in the minutes of the 1992 AARS annual meeting on commodity treatment.

Texas/Mexican Fruit Fly
- Improve Rearing Operations
- Control Microbial Contamination
- Improve Irradiation Techniques
- Develop New Lures and Traps
- Improve Aerial Bait Spray Technology
- Conduct Parasite Trials in Mexico
- Screen Pathogens

Hawaii/Mediterranean Fruit Fly
- Optimize Larval Rearing Operations
- Develop "Naked" Pupation Procedures
- Maximize Egg Survival During Incubation
- Estimate the Probability of Detection
- Determine Feasibility of Mass-Trapping
- Enhance Competitiveness of Released Flies
- Improve Production and Quality Control

Guatemala/Mediterranean Fruit Fly
- Develop Male Annihilation
- Improve Ground and Aerial Bait Sprays
- Advance Sterile Insect Technique
- Develop Integrated Control Methods
- Improve Trap Design and Use
- Evaluate New Attractants
- Improve Quality Control in Mass Rearing

Florida/Caribbean Fruit Fly
- Determine Efficacy of Malathion-Nulure
- Monitor Effects of Irradiation
- Reformulate Larval Diet
- Evaluate Storage of Parasitized Larvae
- Develop Parasitoid Trap

Research needs of APHIS/PPQ and the action taken by ARS to satisfy them are outlined in detail in Appendix C of the 1992 USDA-ARS Action Plan for Fruit Flies Research. This list of needs is supplemented in this report with new and reemphasized 1994 and 1995 priorities for Section C-III, Control/Eradication and Fundamental Biology. Section C-III contains 59 needs for research, 28 submitted by APHIS with 15 as high priority. The following 18 are currently considered to be very important for supporting the action agencies:

## 1994-1995 Priorities Under Section C-III of 1992 Action Plan

### Control and Eradication - General

- Alternative for Aerially Applied Malathion
  High priority need for several years.

- Integrate Biologically-Based Controls
  Need improvements in male annihilation, SIT, augmentative parasite releases, and pathogens. Male only strain, temperature sensitive lethal tests will be conducted in Guatemala in 1994 and 1995.

  Need combinations of controls and a system that is effective and environmentally and socially acceptable. Parasites and parasitoids, male annihilation, female annihilation, and malathion bait spray will be compared with and without a sterile insect component in 1995.

### Chemical Pesticides

- Ground Bait Spray Technology
  Current pesticide use, refinement needed.

- Efficacy of Borax for Bait Spray
  Need to increase speed of action, reduce dosage, and resolve environmental concerns.
  Soil treatments necessary, improvement needed.

## Male/Female Annihilation

- Male Annihilation for Medfly. Need 1995 trials.
- Research for Registration of Chemicals for Male Annihilation
  Need capability for many fruit fly species.
- Female Annihilation for Medfly
  Need to use in combination with other technologies.

## Autocidal Control

- Identify Larval Nutritional Needs in Artificial Diets
  Needed for Medfly and Mexican fruit fly. Sterile fly production is highly variable and prone to diet contamination. Stable production is needed to ensure adequate quality and quantity.
- Standard Diets for Fruit Fly Rearing
  Immediate need is for Medfly and Mexican fruit fly. This will support more precise contract specifications for procurement of diet ingredients.
- Rearing and SIT Technology
  Immediate need is for *Bactrocera latifrons* and *Anastrepha obliqua.*
- More Efficient Rearing
  Need must be accomplished without sacrificing quality and quantity. Great potential for reducing costs by making progress in recycling or disposing of rearing media and in using less expensive diet materials.
- Improved Method of Marking Adults
  Need rapid screening, automated identification, nontransferable in traps, accurate, no adverse impacts on fly quality.
- Quality Control Methods for Field-Released Flies
  Need methods to evaluate behavioral quality of flies and overall success of SIT programs.
- Determine Mating Between Wild Females and Sterilized Males
- Sterile Male-only Release Technique (New high priority need)

## Biological Control

- Parasitoids to Suppress Fruit Fly Populations
  Especially needed for Medfly.
- Effective Parasitoids for Medfly
  Need field trials.
- Quality Control for Parasitoids and Pathogens of Medfly
  Need methods for rearing, packaging, transporting, and releasing natural enemies. Need field trials.

Methods Development depends on the strong, comprehensive research program maintained by ARS, particularly the discovery of new information and new approaches for detecting and controlling fruit flies. It is also imperative that effective partnerships be formed among ARS, APHIS, and our cooperators to extend work across the continuum from basic research to its applications. These partnerships are necessary because the technology development process is dynamic, requiring interdependence and constant exchange to focus resources where they are needed over time. Thus, linkages between research and action in fruit fly programs must continue to be enhanced.

Investigator's Name: **Robert W. Berninger**
Affiliation and Location: **USDA, APHIS, PPQ, MD**
Research and Implementation Areas: **II.A.1.c.0.1., II.A.1.d**
Dates Covered by Report: **1/1/92 - 6/30/93**
Manuscript Codes (see Appendix A): **None**

**SUMMARY:**

**II.A.1.c.0.1** Trained more than 30 PPQ Officers in certifying hot water treatment facilities for mangoes. The Officers are also trained in supervising the treatment.

**II.A.1.d** New equipment for monitoring temperatures of fruit cold treated in transit is constantly being tested and approved for field use where appropriate.

**FY 93 WORK PLANS:**

**II.A.1.c.0.1** Training will be continued by having the PPQ Officer assigned to the foreign facility for supervision of the treatment arrive at the same time the Hoboken Specialist performs the certification and supervision of the treatment.

**II.A.1.d** New sophisticated equipment is being submitted for approval with improved reliability. The acceptance by industry will ensure greater accuracy in PPQ treatments. Testing will continue as new equipment is developed.

Investigator's Name: **Derrell L. Chambers**
Affiliation and Location: **USDA, APHIS, PPQ, MD, GMDS**
Research and Implementation Areas: **I.B.1.a., I.C.1.a,b., I.D.2**
Dates Covered by Report: **1/1/92 - 6/30/93**
Manuscript Codes (see Appendix A): **146,147**

**SUMMARY:**

**I.B.1.a** Developed dose-response data for a variety of TML dispensers to perfect sticky traps for Medfly.

**I.C.1.a,b** Tested additives for Nulure/McPhail traps which increase attractiveness.

**I.D.2** Collaborated with ARS on dry traps for Anastrepha species.

**FY 93 WORK PLANS:**

**I.B.1.a** Our own and industry formulations will be tested to develop specifications for short - and long - term trapping tactics with existing and new trap designs.

**I.C.1.a.b** Field tests will be conducted to identify the utility of added ammonia and citrus oils in bait solutions.

**I.D.2** Concentrated proteins partially purified compounds and synthetic compounds will be field tested in dry and sticky traps for attracting Medfly and *Anastrepha* species.

Investigator's Name: **Derrell L. Chambers**
Affiliation and Location: **USDA, APHIS, PPQ, MD, GMDS**
Research and Implementation Areas: **III.A.2.c., III.B.1.d.0.1., III.C.1.d., III.C.2.a, III.C.2.b.0.3**
Dates Covered by Report: **1/1/92 - 6/30/93**
Manuscript Codes (see Appendix A): **146,147**

**SUMMARY:**

**III.A.2.c** Tested means of enhancing attraction to ground-sprayed sites by adding TML to spray or with sticky panels; and also testing enhancements of bait sprays with stabilized ammonium and lemon oil formulations.

**III.B.1.d.0.1** Developed sticky panels for male annihilation.

**III.C.1.d** Participating in developing and testing temperature sensitive strain for males-only release.

**III.C.2.a** Evaluating improved and alternative quality control tests.

**III.C.2.b.0.3** Participated in MOSCAMED field tests of fly quality.

**FY 93 WORK PLANS:**

**III.A.2.C** Field tests will continue to identify appropriate formulations.

**III.B.1.d.0.1** Sticky traps with long-term effectiveness will be lab and field-tested.

**III.C.1.d** A large field cage will be constructed and fly competitiveness, behavior, longevity and other Quality Control (QC) factors studied.

**III.C.2.a** The use of very large field cages for QC will be evaluated.

**III.C.2.b.0.3** Continue participation in and evaluation of annual field-cage tests.

Investigator's Name: **Derrell L. Chambers**
Affiliation and Location: **USDA, APHIS, PPQ, MD, GMDS**
Research and Implementation Areas: **IV.A.5**
Dates Covered by Report: **1/1/92 - 6/30/93**
Manuscript Codes (see Appendix A): **146,147**

**SUMMARY:**

**IV.A.5** Studying diurnal and daily responses of flies to attractants to improve trapping strategies.

**FY 93 WORK PLANS:**

**IV.A.5** Behavior of flies with respect to response to traps will be studied in large field cages and open field.

Investigator's Name: **Kenneth L. Esau**
Affiliation and Location: **USDA, APHIS, PPQ, MD, MMDC**
Research and Implementation Areas: **III.D.2.b,c.0.4**
Dates Covered by Report: **1/1/92 - 6/30/93**
Manuscript Codes (see Appendix A): **152**

**SUMMARY:**

**III.D.2.b,c.0.4** A laboratory colony of *D. longicaudata* is maintained at a size capable of producing ca. 50,000 parasites per week for field release. Innundative releases have been made in a wild host (yellow chapote) and in citrus in northern Mexico. Parasitism rates of about 35% have been realized.

**FY 93 WORK PLANS:**

**III.D.2.b,c.0.4** Releases will continue in other areas of northern Mexico and attempts will be made to determine if the parasite has become established in last year's release sites.

Investigator's Name: **Danny B. Gates**
Affiliation and Location: **USDA,APHIS, PPQ, MPMC**
Research and Implementation Areas: **I.C.1.a, I.D.1**
Dates Covered by Report: **10/1/91 - 6/30/93**
Manuscript Codes (see Appendix A): **151**

**SUMMARY:**

**I.C.1.a** Recently completed a test comparing the attractancy of Nulure, Torula yeast and a new bait, Argo Steepwater, for MFF. Argo caught from 84% to 22% more flies than other batches of Nulure.

**I.D.1** Completed a small scale preliminary test with 1,000 yellow sticky panels per $mi^2$. Results indicate that we could expect this trapping array to catch at least one of two males > 50% of the time; also working cooperatively with Hercon Corporation in the development of a new design sticky panel trap that in preliminary tests caught 37% more flies than the sticky trap presently in use.

**FY 93 WORK PLANS:**

**I.C.1.a** Testing will continue with APHIS-Guatemala; APHIS-Florida; and ARS-Florida.

**I.D.1** Preparing for a large scale test in California using the sticky panel trap at from 100 - 1,000 per $mi^2$ to satisfactorily analyze the degree of population suppression and/or detection probability at various population densities. Three hundred new design panel traps have been manufactured and are ready for large scale comparisons with the standard.

Investigator's Name: **Danny B. Gates, David R. Lance**
Affiliation and Location: **USDA, APHIS, PPQ, MPMC & HMDS**
Research and Implementation Areas: **I.D.1, I.D.4, I.D.6**
Dates Covered by Report: **10/1/91 - 6/30/93**
Manuscript Codes (see Appendix A): **151**

**SUMMARY:**

**I.D.1** The relative effectiveness of various traps for Mediterranean fruit flies was evaluated in California and Hawaii using releases of marked sterile flies. Yellow sticky panels were more efficient than "bucket type" (e.g., Mission) traps, which were, in turn, slightly more efficient than Jackson traps.

**I.D.4** Sensitivities of operational Mediterranean fruit fly detection and delimitation systems were evaluated in California using release-recapture techniques. The resulting data were used to develop simple probability models, which indicated that the trapping systems currently in use in California are appropriate and adequate for their intended purposes.

**I.D.6** A release device developed in Spain was compared with AgriSense trimedlure plugs and found to be inadequate for use in detection programs for Mediterranean fruit flies.

**FY 93 WORK PLANS:**

**I.D.1** Initiate further evaluations of new panel traps in California.

**I.D.4** Initiate further studies to determine whether the current Mediterranean fruit fly delimitation system or similar trapping system is sufficiently effective for use as a Male Annihilation tool.

**I.D.6** No further work.

Investigator's Name: **Danny B. Gates**
Affiliation and Location: **USDA, APHIS, PPQ, MD, MMDC**
Research and Implementation Areas: **III.A.2.C.0.1., III.A.2.d**
Dates Covered by Report: **1/1/92 - 6/30/93**
Manuscript Codes (see Appendix A): **151**

**SUMMARY:**

**III.A.2.C.0.1** In cooperation with USDA and FDFA in Florida carried out preliminary tests that indicate that malathion concentration in bait sprays can be reduced from 20% to 10% or lower.

**III.A.2.d** Have conducted preliminary tests on the use of a new attractant as the bait when used in aerial applications for fruit flies.

**FY 93 WORK PLANS:**

**III.A.2.C.0.1** In cooperation with Methods Development - Gainesville and Florida Division of Plant Industry will repeat previous tests with bait spray applications for Caribfly using larger droplet sizes and reduced concentrations of Malathion.

**III.A.2.d** In cooperation with Aircraft and Equipment Operations, Mission, Texas, will be conducting a test to compare the efficacy of Nulure and Argo Steepwater when used as the attractants in Mexfly bait spray applications.

Investigator's Name: **Albert W. Guenthner**
Affiliation and Location: **USDA, APHIS, PPQ, MD, MMDC**
Research and Implementation Areas: **III.C.1.d.0.2**
Dates Covered by Report: **1/1/92 - 6/30/93**
Manuscript Codes (see Appendix A): **None**

**SUMMARY:**

**III.C.1.d.0.2** Working on a technique to develop a genetic sexing system (male only) for Mexican fruit fly. Utilizing genetic methodology on cyromazine (IGR)-resistant flies to selectively kill non-resistant females during the larval stage by exposing them to a diet containing cyromazine. Attempting to induce the Y-autosome translocation in the resistant males by exposing them to Gamma radiation. Some progress has been made in developing resistance to cyromazine in diet.

**III.C.3.a.0.1** Investigating the impact of substerilizing treatments (< 7 KR) on parental Mexican fruit fly males and their subsequent offspring. Determining the quality parameters of the surviving $F_1$ and $F_2$ progeny. The adverse effect from the parental male exposure (6 KR) was significantly reduced by the $F_2$ generation.

**FY 93 WORK PLANS:**

**III.C.1.d.0.2** Continue to experiment with various concentrations of cyromazine and levels of Gamma radiation in order to most effectively attain the study's objectives.

**III.C.3.a.0.1** More testing is planned utilizing lower dosages of radiation (3-4-5 KR) on the parental male.

Investigator's Name: **Timothy C. Holler**
Affiliation and Location: **USDA, APHIS, PPQ, MD, FMDS**
Research and Implementation Areas: **I.C.1.a., I.D.2**
Dates Covered by Report: **1/1/92 - 6/30/93**
Manuscript Codes (see Appendix A): **148,150,154**

**SUMMARY:**

**I.C.1.a** In cooperation with ARS, currently evaluating 4 solutions of Argo Steepwater buffered with different percentages of Borax. These are being compared with a

10% solution of Nulure (3% Borax) and the standard Torula yeast/Borax tablet. Results to date suggest that 10% steepwater with 1% Borax is significantly more attractive than all formulations as the bait ages. Steepwater was most attractive at a neutral pH, thus reducing the need for large amounts of buffering agents.

**I.D.2** In cooperation with the USDA-ARS Insect Behavior Group, Gainesville, Florida, actively involved in a test to replace the wet trap (McPhail/Torula yeast) with a dry trap similar to that tested for Medfly in Guatemala. Presently, traps with and without various color bands are being tested with a base attractant and supplemented with "volatiles" attractive to Caribfly. Initial results from these comparisons are inconclusive due to limited numbers of Caribfly trapped. Tests will resume as the adult fly population increases. Some chemistry work remains necessary to provide optimum trap efficacy prior to resumption of testing.

**FY 93 WORK PLANS:**

**1.C.1.a** Work will include expanding tests comparing 10% Steepwater/1% Borax against the standard bait and a solution of Steepwater with sodium hydroxide (to control pH drift). Additionally, a 5, 10 and 20% Steepwater solution will be tested.

**I.D.2** Tests are scheduled to resume in late August or early September as common guava fruit mature. ARS will hire personnel and Methods personnel will supervise trap servicing functions. Work will be accomplished in the Ft. Pierce, Florida vicinity.


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| Investigator's Name: | **Timothy C. Holler** |
| Affiliation and Location: | **USDA, APHIS, PPQ, MD, FMDS** |
| Research and Implementation Areas: | **II.A.2.a.0.1** |
| Dates Covered by Report: | **1/1/92 - 6/30/93** |
| Manuscript Codes (see Appendix A): | **148,150,154** |


**SUMMARY:**


**II.A.2.a.0.1** In cooperation with ARS Gainesville, Florida, assistance in small field plot tests utilizing gibberellic acid/surfactant. The objective of these tests is to develop a treatment which provides for delayed oviposition by Caribfly until most fruit host has been harvested. Preliminary field-cage test results from grapefruit treated with gibberellic acid (and a surfactant) indicate that the treated fruit had up to 90% fewer Caribfly than untreated fruit. These results suggest that this treatment can extend by at least 2 months the fruits inherent resistance to fruit flies when applications are made just before the fruit turns from green to orange or yellow.


**FY 93 WORK PLANS:**

**II.A.2.a.0.1** No field-cage test activity is scheduled for FY 93. However, as part of Phytotoxicity studies with ARS Horticultural Laboratory in Orlando, Florida, will assist in the determination of which ground equipment is best suited for gibberellic acid/surfactant grove application.

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| Investigator's Name: | **Timothy C. Holler** |
| Affiliation and Location: | **USDA, APHIS, PPQ, MD, FMDS** |
| Research and Implementation Areas: | **III.D.2.C.0.4** |
| Dates Covered by Report: | **1/1/92 - 6/30/93** |
| Manuscript Codes (see Appendix A): | **148,150,154** |

**SUMMARY:**

**III.D.2.C.0.4** In cooperation with the Florida Department of Agriculture and Consumer Services - Division of Plant Industry, assisting in the establishment of a parasite augmentation project in a three county area of "southeast" (Ft. Pierce vicinity) Florida. First releases at selected sites near commercial grove infestations are tentatively scheduled for the first week of June 1993. As of August 8, 1993, ca. 6 million parasitoids have been released in a 3 county area. Results from a pre-parasite release survey indicate that 2 parasitoid species exist. The parasitization rate ranges from 1.6 to 5.9 percent. Post release fruit collection/inspection has been initiated.

**FY 93 WORK PLANS:**

**III.D.2.C.0.4** Continue organizing fruit collection/inspection for parasite efficacy determination following release. Continue ADODR responsibilities in monitoring "Research" aspect of Parasite Control Program in 3 county area of "Southeast" Florida. Study parasite periodicity (egg laying time)/fecundity/ longevity at the laboratory level and apply findings to field situation.

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| Investigator's Name: | **David R. Lance** |
| Affiliation and Location: | **USDA, APHIS, PPQ, MPMC & HMDS** |
| Research and Implementation Areas: | **III.C.1., III.C.2.A., III.C.2.b.0.4** |
| Dates Covered by Report: | **10/1/91 - 6/30/93** |
| Manuscript Codes (see Appendix A): | **151** |

**SUMMARY:**

**III.C.1** Developed data on factors promoting pupation of mass-reared Mediterranean fruit flies in the absence of a medium such as vermiculite. Results indicate that "naked" pupation may be feasible for mass-rearing operations if larvae can be thoroughly dried, held in complete darkness, and exposed to an airflow. Collaborator: McInnis (ARS).

**III.C.2.a** Initiated the development of quantitative analysis of courtship behavior as a quality evaluation tool for mass-reared Mediterranean fruit flies. Video techniques were developed, and standardized data were collected using irradiated and unirradiated laboratory-reared flies. Data collection was initiated using P-generation wild flies.

**III.C.2.b.0.4** Field-cage studies were used to evaluate mating competitiveness and compatibility of mass-reared, sterile Mediterranean fruit flies with wild-type flies. Declines in compatibility of sterile vs. wild flies from Kauai but not from other islands indicate that evolved resistance to SIT is (currently, at least) the most likely explanation for the poor performance of sterile flies during the ARS pilot SIT project on Kauai. Current shipping, handling and release procedures appeared to

cause slight but measurable declines in mating competitiveness. Collaborator: McInnis (ARS).

**FY 93 WORK PLANS:**

**III.C.1** Evaluate effects of temperature and humidity on "naked" pupation if and when environmentally-controlled space becomes available.

**III.C.2.a** Continue evaluations to determine if ethograms and other quantitative representations of courtship behavior can be used to predict mating compatibility of various strains of Mediterranean fruit flies.

**III.C.2.b.0.4** Conclusively demonstrate that resistance to SIT is or is not present in Kauai populations; if it is, investigate possible mechanisms. Initiate studies to determine the relative effectiveness of a genetic sexing (tsl) strain in Guatemala.

Investigator's Name: **Adelaido J. Martinez**
Affiliation and Location: **USDA, APHIS, PPQ, MD, MMDC**
Research and Implementation Areas: **I.D.2**
Dates Covered by Report: **1/1/92 - 6/30/93**
Manuscript Codes (see Appendix A): **152**

**SUMMARY:**

**I.D.2** Field studies involving bacterial metabolites indicated that they are as good as protein baits in attracting *A. ludens* adults. The same test conducted in Guatemala (Romeo Martinez) indicated that the metabolites attract various *Anastrepha* spp.

**FY 93 WORK PLANS:**

**I.D.2** Field testing of bacterial metabolites will continue in Guatemala.

Investigator's Name: **Adelaido J. Martinez**
Affiliation and Location: **USDA, APHIS, PPQ, MD, MMDC**
Research and Implementation Areas: **III.A.2.a**
Dates Covered by Report: **1/1/92 - 6/30/93**
Manuscript Codes (see Appendix A): **152**

**SUMMARY:**

**III.A.2.a** *Bacillus thuringiensis* (B.t.) isolates tested against *A. ludens* adults have shown good activity (> 70% mortality in 10 days) in the laboratory. *Beauvaria bassiana*, a fungal pathogen isolated from boll weevils has been shown to kill *A. ludens* adults in the laboratory.

**FY 93 WORK PLANS:**

**III.A.2.a** Further B.t. isolations are planned in Guatemala with a goal of 50-75 B.t. cultures to be isolated and tested. Other *B. bassiana* isolates will be tested in the laboratory.

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| Investigator's Name: | **Douglas C. Prasher** |
| Affiliation and Location: | **APHIS, PPQ, Otis MDC, ANGB, MA** |
| Research and Implementation Areas: | **I.F** |
| Dates Covered by Report: | **10/1/91 - 6/30/93** |
| Manuscript Codes (see Appendix A): | **None** |

**SUMMARY and FY 93 WORK PLANS:**

**I.F.** Initiated studies to examine the genetic relationship among the Medflies intercepted in California as well as between the California specimens and feral populations found in other geographic locations (e.g. S.A., Central A., Hawaii, etc.). DNA sequences of mitochondrial DNA loci will be compared.

A Germplasm Repository is being established at Otis MDC so scientists studying population genetics of the Medfly can share specimen materials and information. All specimens intercepted within the continental U.S. are being deposited within the Repository. Medfly specimens for deposit are also being requested from feral populations outside the continental U.S. (South and Central America, Mediterranean countries, Africa, western Australia, and Hawaii) and laboratory strains in culture.

Initiated a study to predict the geographic origin of Medflies trapped in California and the relatedness of flies trapped in separate geographic locations in California. This project will attempt to identify geographic strains of the Mediterranean fruit fly on the basis of nucleotide substitutions within the internal transcribed spacer (ITS) regions of the ribosomal RNA gene clusters. Cooperator: Bruce Campbell, ARS, Albany, CA.

Initiated a study to identify a set of genetic markers using RAPD PCR that can be used to determine the source of origin of Medflies in California. In addition, the same genetic markers can be used to determine the relatedness of flies captured in different infestations in California. Cooperator: David Haymer, University of Hawaii at Monoa, Honolulu, HI.

Initiated a study to identify genetic markers that will aid in determining the source of intercepted Medflies in California. The genetic variation discovered in these studies is expected to address the issue of whether the various Medfly infestations in southern and central California have been the result of multiple introductions or a single colonization event. A series of genetic markers based on simple sequence length polymorphisms (SSLP) will be developed in this project. Cooperator: Bruce McPheron, Pennsylvania State University, University Park, PA.

Initiated a study to develop PCR-based methodologies to analyze polymorphic restriction sites in Medfly mitochondrial DNA. The techniques developed will be used to predict the geographic origin of Medflies trapped in California as well as the relatedness of flies trapped in separate geographic locations within California. Cooperator: W. Steve Sheppard, ARS, Beltsville, MD.

A contract has been awarded for the collection of Mediterranean fruit fly specimens in foreign locations. These specimens will be deposited into the Medfly Germplasm Repository for distribution to scientists studying population genetics of the Medfly. Contractor: Gary Steck, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, FL.

## OVERVIEW AND RECOMMENDATIONS/APHIS PRIORITY NEEDS

### Overview

Over the years the USDA has strived to maintain a balanced program on fruit flies and on the management of fruit fly problems in its research, development, education, regulatory and action programs. As the "in-house" research agency for USDA, ARS and its cooperators have accelerated their research efforts on fruit flies in order to better address issues related to (a) detection and delimitation, (2) exclusion, (3) control and eradication, and (4) fundamental biology of the key fruit fly pests. This research effort is aimed at halting the geographical spread of these pests; providing for the safe movement in commercial agriculture of fruits and vegetables out of areas already invaded; reducing costs and damage to agriculture in those invaded areas; and, finally, developing environmentally sound, publicly-acceptable, and economically efficacious systems to suppress or eradicate established populations of fruit flies.

In FY 93 ARS devoted $9.45 million to tephritid fruit fly research. Of this $9.45 million, $3.02 million is for research on Mediterranean fruit fly which is undertaken at four ARS locations and involves nine projects. Those four locations are Gainesville, FL ($77,200); Beltsville, MD ($124,000); Hawaii ($2.76 million); and Albany, CA ($56,800). Most of the ARS Medfly effort is concentrated in Hawaii, although the efforts of the other locations are vital and important team components to the overall Medfly program between the ARS scientists themselves and with the cooperators. ARS research also covers a variety of other fruit fly pests, such as Caribbean at $1.32 million (Gainesville, Orlando, and Miami); Malaysian at $745,900 (Beltsville & Hawaii); melon at $793,100 (Hawaii); Mexican at $912,500 (Gainesville and Weslaco); oriental at $1.04 million (Beltsville & Hawaii); papaya at $154,500 (Miami and Hawaii); and other fruit flies at $1.45 million (Miami, Beltsville, Hawaii, Weslaco, and Fresno), such as the West Indian, *Rhagoletis* sp. (e.g., walnut husk fly), *Anastrepha serpentina, A. striata, A. distincta*, etc. Total funds devoted to fruit flies other than Medfly were $6.43 million in FY 93.

Focus of the ARS research over the past few years has centered on addressing the four major program areas outlined in the National Action Plan that was developed in the summer of 1991. For example, ARS is cooperating with APHIS in the evaluation of the male attractant, ceralure, on wild Medfly populations in Guatemala. This includes the evaluation of differing types of traps, including yellow sticky panels, and the incorporation of ceralure into stickum; ARS scientists are also working closely with industry on formulation. In addition to the progress with ceralure, a test of commercial polymer panels impregnated with trimedlure has shown high and prolonged activity after nearly 3 months in the field. Captures of male Medflies at day 71 were over twice that in the standard Jackson trap baited with a fresh plug dispenser. Polymer plug dispensers of ceralure were also evaluated in the field and found to be more attractive than trimedlure after 3 to 4 months. Also, an attracticide system similar to that tested on Caribfly was attractive to female Medflies in Guatemala. ARS is also developing a procedure to analyze environmental samples for trace ceralure levels in plots treated with the attractant.

Also, ARS scientists at Beltsville and scientists at Pennsylvania State University are working cooperatively on efforts to find molecular markers that can be used to pinpoint sources of Medfly infestations. RFLP's were used to survey 15 established natural populations and 5 laboratory cultures from Hawaii, Central and South America, West Africa, and the recent California infestations. Hawaii was found to be an unlikely source for the 1989 and 1991 infestations in California. ARS scientists and scientists at the University of Hawaii are also using DNA analysis with emphasis on genomic DNA in this source determination effort.

ARS scientists at Gainesville, FL, in close cooperation with scientists in Guatemala, have been focusing on quality of mass-reared Medflies for the sterile male program. This research has also

been aimed at Medfly pheromone and other attractants to determine the minimal pheromone components necessary for attraction of female Medflies. The studies, carried out in Guatemala, should lead to the development of a pheromone-based system to monitor and suppress Medfly populations. Female attractant chemicals work has led to the development of a food-based dry trap.

Additionally, ARS scientists in Albany, CA are cooperating with ARS scientists in Hawaii on the chemistry and major/minor components of the Medfly female attractant pheromone. There have been a large number of components identified for the Medfly female attractant pheromone; five of these components constitute about 95% of the pheromone material. Although the Medfly pheromone does not have similar effectiveness as the lepidopteran pheromones, it should be useful for monitoring, and maybe for use in the Hawaiian eradication program. Work is also underway which indicates that ammonia attracts female Medflies; slow release of ammonia from the protein bait could be a major breakthrough for the survey and control of Medflies. It has been shown that the female walnut husk fly is strongly attracted to ammonia.

ARS scientists have also identified several lines of lemon and pummelo that are highly resistant to Medfly throughout the growing season. Resistant factors, once identified, might be useful in plant breeding and gene transfer programs.

In Hawaii, there are two overall major ARS efforts: Research to support the eradication program and quarantine treatment research. In terms of the eradication research effort, focus is on attempts to improve existing attractants and to identify new attractants for male and female Medflies, and on the sterile insect technique (SIT) with efforts aimed at improving mass-rearing technology and a males-only strain. Another emphasis area is on biocontrol using parasitoids such as the *Diachasmimorpha longicaudata* and *Psyttalia fletcheri* species. The attractants research has included fine tuning and improvement of the ceralure system. This research team's work, in cooperation with ARS scientists in Beltsville, as mentioned above, has resulted in the incorporation of ceralure into slow release panels coated with stickum. The team has also been involved with the identification of the attractant materials in protein hydrolysate. An ARS scientist in Hawaii has determined that the pH of the protein bait plays an important role in trap captures of both male and female Medfly; thus, it is important that a system for maintaining the proper pH be employed. Another ARS scientist in Hawaii is also working on pheromone attractants for the female, as well as attractants from fruit odors. The cooperative efforts between ARS scientists in Hawaii, Albany and Gainesville have resulted in the identification of a large number of components in the pheromone from the Medfly male; five synthetic materials have been tested in the laboratory, which attract up to 50% of a test population. It's likely that the minor components will be important and the components of both the pheromone and host-fruit odors will influence female behavior.

The ARS Hawaii research group has also undertaken a sterile insect (SIT) pilot test in commercial coffee on Kauai. Release rates have been at 1 million sterile flies/sq. mile over 25 miles for a six-month period. This failed to suppress wild Medfly populations and behavioral studies have uncovered some problems in sterile flies released from aircraft. Plans are to include augmentation with parasites in the pilot test. This method was tested in Maui by ARS where some reduction was demonstrated using parasites to lower the populations, followed by SIT.

Another ARS scientist in Hawaii, working with a pupal color sexing strain of Medfly allowing for a males only release, has shown significant promise in reducing wild fly populations in field tests on commercial coffee. Additionally, Hawaiian scientists are working on the parasitoid aspect of the program with oriental fruit fly and Medfly. The pilot test on Maui to evaluate parasitoid augmentation, primarily for the Medfly, might be used as a predictive measure of augmentation for Medfly control in larger-scale operations for commercial coffee. Current work also is focused on improving mass rearing of parasitoids, general parasitoid biology, and identifying factors involved in longevity, and dispersal of parasitoids.

ARS has an intensive program in quarantine treatment research, especially in the use of non-chemical treatments. ARS is committed to developing methodology to address the possible loss of registration for methyl bromide. The quarantine treatment research for Medfly involves validation of the effectiveness of quarantine treatments on a wide variety of Medfly hosts. Heat and cold treatments are a major focus in addition to hot water dips/high temperature. Successful cold treatments were devised for litchi and carambola against the Medfly. Although heat and cold treatments seem to be the most promising at this time, the success of the treatment depends on the commodity; for example, tropical fruits tend to take heat treatment, but are more prone to chilling injury. Modified atmospheres and irradiation are other possibilities for examination. ARS laboratories in Orlando, Fresno, Hilo, and Weslaco carry out the fruit quality component of the overall ARS fruit fly program relative to quarantine treatment protocols.

Other significant work and accomplishments include: development of a new commercial polymer dispenser for trimedlure; development of successful temperature treatments for a variety of tropical and subtropical fruit for the major fruit fly pests of Hawaii, Florida, and Texas, and for imports from Latin America and the Caribbean basin; the use of gibberellic acid treatments for citrus against Caribfly and Mexican fruit fly; development of an artificial diet for *Anastrepha obliqua*; and mass releases of a parasite that substantially reduces Caribfly populations.

## Recommendations

The following recommendations needing further research consideration were generated during the workshop and are considered as very high priority:

1. Optimization of mass rearing/SIT/quality control/diet recycling.
2. Development of methodology to control fruit flies entirely with biologically-based technologies.
3. Elimination of dependence on malathion in bait sprays.
4. Development and evaluation of a temperature-sensitive lethal strain of fruit fly.
5. Development of commodity treatments to replace methyl bromide.
6. Determination of the precise origin and pathway entry of exotic fruit fly species into the U.S./prediction/prevention.
7. Development and pilot testing of technologies for the eradication of the four major fruit fly species in Hawaii.
8. Development of highly effective attractants and trapping systems for all economically significant fruit fly species.
9. Development of fly-free zone technology.
10. Generation of increased knowledge on fruit fly behavior and field biology.

Note: Fruit flies identified of particular high priority are Medfly, *Anastrepha* sp., oriental fruit fly, and melon fly.

## APHIS Priority Needs

A list of 18 very high priority needs were submitted by APHIS and are further detailed in section VII of this report:

1. Alternative for aerially-applied malathion.
2. Integration of biologically-based controls, especially in terms of improvements in male annihilation, SIT, augmentative parasite releases, and pathogens.
3. Ground bait spray technology.
4. Efficacy of borax for bait spray.
5. Male/female annihilation.
6. Research for registration of chemicals for male annihilation.
7. Female annihilation for Medfly in combination with other technologies.
8. Identify larval nutritional needs in artificial diets, especially for Medfly and Mexican fruit fly.
9. Standard diets for fruit fly rearing, especially for Medfly and Mexican fruit fly.
10. Rearing and SIT technology, especially for *Bactrocera latifrons* and *Anastrepha obliqua*.
11. More efficient rearing.
12. Improved method of marking adults which is needed for rapid screening, automated identification, and which is nontransferable in traps, accurate, and with no adverse impacts on fly quality.
13. Quality control methods for field-released flies and methods to evaluate behavioral quality of flies and overall success of SIT programs.
14. Method to determine mating between wild females and sterilized males.
15. Sterile male-only release technique.
16. Parasitoids to suppress fruit fly populations, especially Medfly.
17. Effective parasitoids for Medfly along with field trials.
18. Quality control for parasitoids and pathogens of Medfly with emphasis on methods for rearing, packaging, transporting, and releasing natural enemies, as well as a need for field trials.

## POST-WORKSHOP COMMENTS/MISCELLANEOUS RECOMMENDATIONS

July 26, 1993

SUBJECT: Mini-Review of the USDA-ARS Action Plan for Fruit Fly Research

TO: Fruit Fly Researchers

FROM: James R. Coppedge, NPL
Applied Entomology

Robert M. Faust, NPL
Fundamental & Molecular Entomology

The subject mini-review was held as scheduled on June 28, 1993, from 8:30 a.m. to 5:00 p.m. in Bldg. 005, Room 021, BARC-West, Beltsville, Maryland. The research summaries and plans provided by ARS researchers were used to prepare a document entitled "Fruit Fly Research: 1993 Supplement to the USDA-ARS Action Plan." This document was available to workshop participants approximately one week prior to the meeting.

The format used for this review was one we had not tried before. The Joint ARS-APHIS Fruit Fly Working Group appointed assessment teams (ARS and APHIS personnel) for each section of the plan with Dr. Faust as the overall coordinator. The assessment teams requested reports from the scientists and developed an overview of the research accomplishments for each of the 4 sections of the National Action Plan since it was drafted.

At the review, the ARS member of the assessment team reviewed the research accomplishments while the APHIS representative reported on how ARS scientists were meeting the technology needs of the action and regulatory agencies. Approximately 28 individuals attended the review representing ARS, APHIS, and the States. There was considerable constructive dialogue during and following these presentations (the minutes from the meeting and a list of attendees will be included in the final review document). Of special interest to the ARS National Program Staff were the comments from the group on how the used format met their needs for information and updates. Some of the more pertinent comments from the group are listed and briefly discussed.

(1) The review participants felt that overall, the review format was good and, when combined with the written report, was very informative. They felt that in the current climate of limited resources, a mini-research review conducted in this manner was appropriate. However, the group recommended that such brief reviews should alternate with reviews which include all of the scientists and a broader representation of the action and regulatory groups.

(2) The group was very complimentary of ARS efforts to focus the fruit fly research programs on the needs of the action and regulatory agencies/and was impressed with the quality of research in ARS, the innovation involved, and the dedication of ARS scientists. They were also complimentary of the review document and felt it was valuable to them especially when they visit ARS laboratories.

(3) The group felt that ARS needed to have more of the scientists at the meeting--especially those doing research where they see the possibilities of near future implementation. They had questions which they felt were not adequately answered because the appropriate resource person was not there.

(4) The group was critical that we did not include the level of SY effort on each project statement which makes it difficult to assess the amount of effort ARS devotes in specific areas of research.

(5) The group was critical that ARS didn't have more of a research presence in Mexico and Central America. ARS has agreed to address this concern during the next few months.

(6) The group commented that the information provided by some research scientists was unclear or inadequate to determine the significance of the reported progress.

We were most concerned with item 6 and felt that some of the write-ups did not reflect the true accomplishments and plans of the scientists. This is a critical point, as the review document provides a convenient means of evaluating the impact of individual and laboratory programs. In view of this, we have decided to give each of you an opportunity to correct, expand, update, etc., your input into this review. We have enclosed a copy of your input for consideration and possible revision as well as instructions on how to provide your revisions. Please note that your report should now cover the period from 10/1/91 to 4/30/93 and this will match the time frame of the publications list. We will incorporate any changes into the final document provided they are received by C.O.B. August 16, 1993. **If we do not receive revisions by that date, we will assume you are comfortable with your contribution as originally submitted.**

Best of luck in your research and thanks for your help with this recent review of the National Fruit Fly Research Program.

Enclosure

cc:

| | | |
|---|---|---|
| E. B. Knipling | H. Brooks | J. Krysan |
| W. Martinez | F. Horn | M. Carter |
| R. Reginato | D. Murrell | J. Fowler |

## APPENDIX A

## PUBLICATIONS LIST
## August 1991 - May 1993

## ARS PUBLICATIONS


1. Avery, J.W., D.L. Chambers, R.T. Cunningham and B.A. Leonhardt. 1992. Use of ceralure attractant for Mediterranean fruit fly: Delivery rate in male annihilation test. 204th National Meeting of the American Chemical Society. San Francisco, CA. (Abstract).

2. Avery, J.W., D.L. Chambers, R.T. Cunningham, B.A. Leonhardt and A.B. DeMilo. 1993. Relative attractancy of ceralure and trimedlure for Mediterranean fruit fly *Ceratitis capitata* (Weidemann). 205th National Meeting of the American Chemical Society. Denver, Colorado. (Abstract).

3. Baker, J.D., R.R. Heath and J.G. Millar. 1992. An equilibrium and stability study of $\Delta^1$-pyrroline. J. Chem. Ecol. 18(9): 1595-1602.

4. Baker, J.D. and R.R. Heath. NMR spectral assignment of the lactone pheromone components emitted by Caribbean and Mexican fruit flies. J. Chem. Ecol. (in press).

5. Baranowski, R., H. Glenn and J. Sivinski. 1993. Biological control of the Caribbean fruit fly, *Anastrepha suspensa* (Loew). Fla. Entomol. (in press).

6. Brown, G.E., W.R. Miller and R.E. McDonald. 1991. Control of green mold in Marsh grapefruit with vapor heat quarantine treatment. Proc. Fla. State Hort. Soc. 104: 115-117.

7. Calkins, C.O. 1991. The effect of mass rearing on mating behavior of Mediterranean fruit flies. Pp.153-160. In: K. Kawasaki, O. Iwahashi and K.Y. Kaneshiro (eds.), Proc. Intl. Symp. on Biology and Control of Fruit Flies. Okinawa.

8. Calkins, C.O. 1993. Future directions in Caribbean fruit fly control. Fla. Entomol. (in press).

9. Calkins, C.O. 1992. Preface. For: Proceedings of the International Symposium on Fruit Flies of Economic Importance, held in Antigua, Guatemala, October 14-20, 1990. Published Dec. 1992.

10. Chang, F., E.B. Jang, C.L. Hsu, M. Ma, and L. Jurd. Benzodioxole-1,3-benzodioxole derivatives and their effects on the reproductive physiology of insects. Arch. Insect Biochem. Physiol. (accepted 2/1/93).

11. Conway, W.S., C.E. Sams, R.G. McGuire and A. Kelman. 1992. Calcium treatment of apples and potatoes to reduce postharvest decay. Plant Dis. 76: 329-334.

12. DeMilo, A.B., J.D. Warthen, Jr., M. Sardashti and J. O'Donnell. 1992. Structure confirmation of the four *trans* isomers of ceralure, a Medfly attractant by NMR. Proceedings, 204th Amer. Chem. Soc. Natl. Mtg., Agrochem. Div. No. 17. (Abstract).

13. DeMilo, A.B., R.T. Cunningham and T.P. McGovern. 1993. Further structure-activity correlations related to trimedlure. Beltsville Symposium XVIII, Pest Management: Biologically Based Technologies. Paper No. 50 (Abstract).

14. DeMilo, A.B., J.D. Warthen, Jr. and B.A. Leonhardt. 1991. A capillary GC method for the analysis of ceralure, a potent and persistent Medfly attractant. ESA Annual Meeting, December 8-12, Reno, Nevada. (Abstract).

15. Epsky, N.D. and R.R. Heath. Effects of food availability on pheromone production by males of *Anastrepha suspensa* (Diptera: Tephritidae). Environ. Entomol. (in press).

16. Epsky, N.D. and R.R. Heath. 1993. Pheromone production by males of *Anastrepha suspensa* (Diptera: Tephritidae) under natural light cycles in greenhouse studies. Environ. Entomol. 22: 464-469.

17. Flath, R.A., E.B. Jang, D.M. Light, T.R. Mon, L. Carvalho, R.G. Binder and J.O. John. 1993. Volatile pheromonal emissions from the male Mediterranean fruit fly: effects of fly age and time of day. J. Agric. Food Chem. (in press).

18. Foote, R.H., F.L. Blanc and A.L. Norrbom. 1993. *Handbook of the Fruit Flies (Diptera: Tephritidae) of America North of Mexico.* Cornell University Press, Ithaca, NY. (publication expected June 25).

19. Greany, P.D. and J.P. Shapiro. 1993. Manipulating and enhancing citrus fruit resistance to the Caribbean fruit fly (Diptera: Tephritidae). Florida Entomologist 76. (in press).

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31. Handler, A.M. 1992. Molecular genetic mechanisms for sex-specific selection. Pp. 11-32. In: *Advances in Insect Rearing for Research and Pest Management* . T.E. Anderson and N.C. Leppla (eds.). Westview Press, Boulder.

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70. Leonhardt, B.A., R.T. Cunningham, D.L. Chambers and J.W. Avery. 1993. Development of dispensing sysetms for Mediterranean fruit fly attractants. 205th National Meeting of the American Chemical Society. Denver, Colorado. (Abstract).

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## APHIS PUBLICATIONS


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147. Avery, J.W., R.T. Cunningham, D.L. Chambers and E.M. Harte. 1993. Controlled-release panel traps for the Mediterranean fruit fly (Diptera: Tephritidae). (Abstract).

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153. Sivinski, J.M., C.O. Calkins, R. Baranowski, D. Harris, J. Bromibila, T. Holler and G. Dodson. 1993. Suppression of a Caribbean fruit fly *Anastrepha suspensa* (Lowe) population through augmented releases of the parasite. (Abstract).

154. Weidhass, D.E., C. Calkins, T.C. Holler, J. Sivinski, R. Baranowski and D. Harris. 1993. A life history computer simulation model of the effect of the release of sterile insects and/or a parasitoid(s) with or without bait sprays on the density of fruit flies. (In review).

## APPENDIX B

## INDEX OF CONTRIBUTORS: BY LOCATION

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## APPENDIX C

## INDEX OF CONTRIBUTORS: ACTION AREA/MANUSCRIPTS

| CONTRIBUTOR'S NAME | SECTION | MANUSCRIPT CODES |
|---|---|---|
| Armstrong, J.W. | II | 21,28,30 |
| Avery, J.W. | I | 1,2,70 |
| Berninger, R.W. | I | None |
| Calkins, C.O. | I,II,III | 7,8,9,51,54,69,118,126,142 |
| Campbell, B.C. | I,IV | None |
| Chambers, D.L. | I,III,IV | 146,147,149 |
| Chan, H.T. | II,III | 41,42,61 |
| Cunningham, R.T. | I,III | 1,2,13,63,64,70,76,143,144 |
| DeMilo,A.B. | I,III | 2,12,13,14,63,64,143,144 |
| Esau, K.L. | III | 152 |
| Flath, R.A. | I | 17,71,112,130 |
| Gates, D.B. | I,III | 151 |
| Gould, W.P. | II | None |
| Greany, P.D. | II | 19 to 23, 118 |
| Guenthner, A.W. | III | None |
| Hallman, G.J. | II | 25 to 30, 90,115 |
| Handler, A.M. | III,IV | 31 to 34, 105,106 |
| Hansen, J.D. | II,III | 35 to 45,49,131 to 133 |
| Harris, E.J. | III | 46 to 48 |
| Heath, R. R. | I,III | 3,4,15,16,51 to 55,67,68,122,123 |
| Hennessey, M.K. | II,III | 56 to 59 |
| Holler, T.C. | I,II,III | 148,150,153,154 |
| Jackson, C.G. | III,IV | None |
| Jang,E.B. | I,II,III,IV | 10,17,60,61,62,71,88 |
| Lance, D.R. | III | 151 |
| Landolt, P.J. | I,IV | 51,54,55,65,66,67,68 |
| Leonhardt, B.A. | I,III | 1,2,14,64,70 |
| Light, D.M. | I,IV | 17,62,71,134 |
| Lindegren, J.E. | III | None |
| Liquido, N.J. | I,II,III,IV | 63,72 to 79 |
| Mangan, R.L. | II, III, IV | 80,81,109,119 |
| Mankin, R.W. | IV | 82 |
| Martinez, A.J. | I,III | 152 |
| Mayer, M.S. | I | None |
| McDonald, R.E. | II | 6,23,89 to103,118 |
| McGuire, R.G. | II | 11,83 to 85 |
| McInnis, D.O. | I, III | 50,86,87 |
| Miller, W.R. | II | 6,89 to 100 |
| Mitcham, E.J. | II | 89,92,95, 101 to 103 |
| Moreno, D.S. | I, III, IV | 81,109 |
| Norrbom, A.L. | I | 18,104,135 |
| Prascher, D.C. | I | None |
| Purcell, M. | III, IV | 128,137 |
| Robacker, D.C. | I | 108 to 113 |
| Seo, S.T. | III | None |
| Sharp, J.L. | II | 27,39,56,58,90,93,114 to 117 |

| | | |
|---|---|---|
| Sheppard, W.S. | I | 120,129 |
| Sivinski, J.M. | I, III, IV | 5,51,54,55,66,121 to 125 |
| Teranishi, R.S. | I | 72,130,134 |
| Thompson, F.C. | I | 135 |
| Vail, P.V. | III | None |
| Vargas, R.I. | III, IV | 24,107,127,128,136 to 141 |
| Waiss, A.C | II | None |
| Warthen, J.D.,Jr. | I | 12,14,143,144,145 |
| Yokoyama, V.Y. | II | None |

## APPENDIX D

## REVIEW OF USDA-ARS ACTION PLAN FOR FRUIT FLY RESEARCH/WORKSHOP

June 28, 1993

Building 005, Room 021
BARC-West
Beltsville, MD 20705

Tentative Program

Morning
MODERATOR: R. M. Faust

| | | |
|---|---|---|
| 8:30 - 8:35 AM | Call to Order and Administrative Details | Robert M. Faust |
| 8:35 - 8:40 AM | Welcome to the Conference | Edward B. Knipling |
| 8:40 - 8:50 AM | Objectives and Charge to the Workshop | Robert M. Faust |
| 8:50 - 9:05 AM | ARS Perspective | James R. Coppedge |
| 9:10 - 9:20 AM | APHIS Perspective | Jerry Fowler |

**Action Plan Review, Plans and Recommendations**

| | | |
|---|---|---|
| 9:20 - 10:35 AM | I. Detection and Delimitation<br>• Program Summary<br>• Workplans/assessments<br>• Priorities, needs, and recommendations | B. Leonhardt/M. Holmes Leaders |
| 10:35 - 10:45 AM | Break | |
| 10:45 - 12:00 PM | II. Exclusion<br>• Progress summary<br>• Workplans/assessments<br>• Priorities, needs, and recommendations | R. Mangan/P. Gomes Leaders |
| 12:00 - 1:15 PM | Lunch | |

Afternoon
MODERATOR: J. Krysan

| | | |
|---|---|---|
| 1:15 - 2:30 PM | III. Control and Eradication<br>• Progress summary<br>• Workplans/assessment<br>• Priorities, needs, and recommendations | E. Jang/D. Lance Leaders |
| 2:30 - 2:45 PM | Break | |

| | | | |
|---|---|---|---|
| 2:45 - 4:00 PM | IV. Fundamental Biology<br>• Program summary<br>• Workplans/assessments<br>• Priorities, needs, and recommendations | E. Jang/D. Lance | Leaders |
| 4:00 - 4:15 PM | Workshop Assessment/Future Workshops | J. Coppedge | |
| 4:15 - 4:30 PM | Closing Remarks | C. Schwalbe | |

ADJOURN

## APPENDIX E

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**June 28, 1993**

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## APPENDIX F

### Common and Scientific Names for Tephritid Fruit Flies

| Common Name | Scientific Name |
|---|---|
| Mediterranean Fruit Fly | *Ceratitis capitata* |
| Oriental Fruit Fly | *Bactrocera* (Dacus) *dorsalis* |
| Melon Fly | *Bactrocera* (Dacus) *cucurbitae* |
| Malaysian Fruit Fly | *Bactrocera* (Dacus) *latifrons* |
| Mexican Fruit Fly | *Anastrepha ludens* |
| Caribbean Fruit Fly | *Anastrepha suspensa* |
| West Indian Fruit Fly | *Anastrepha obliqua* |
| South American Fruit Fly | *Anastrepha fraterculus* |
| "Guava" Fruit Fly | *Anastrepha striata* |
| Walnut Husk Fly | *Rhagoletis completa* |
| Papaya Fruit Fly | *Toxotrypance curvicauda* |

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